

Figure 1

TRAIL induced apoptosis in Jurkat Cells
12 HOURS

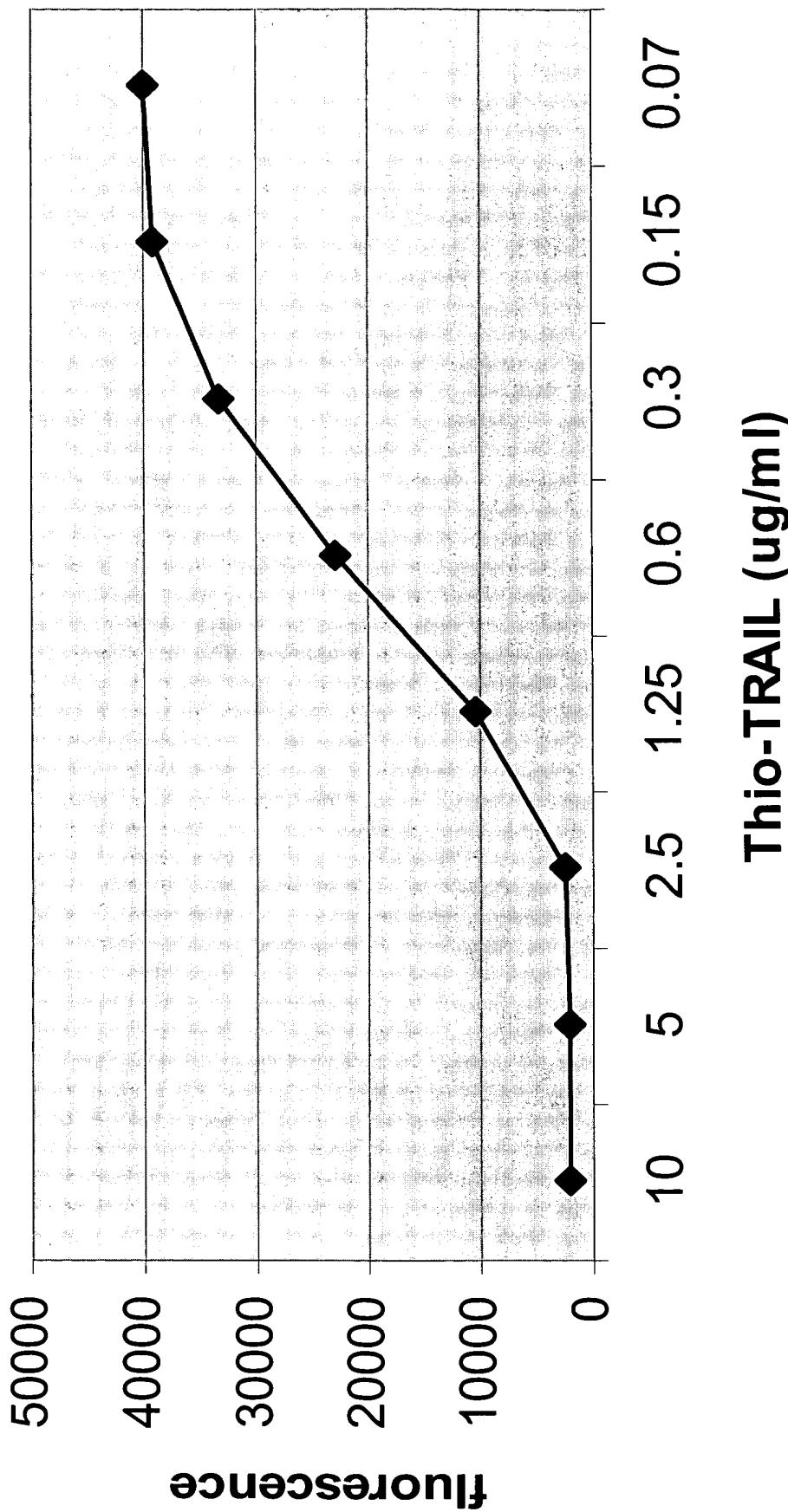
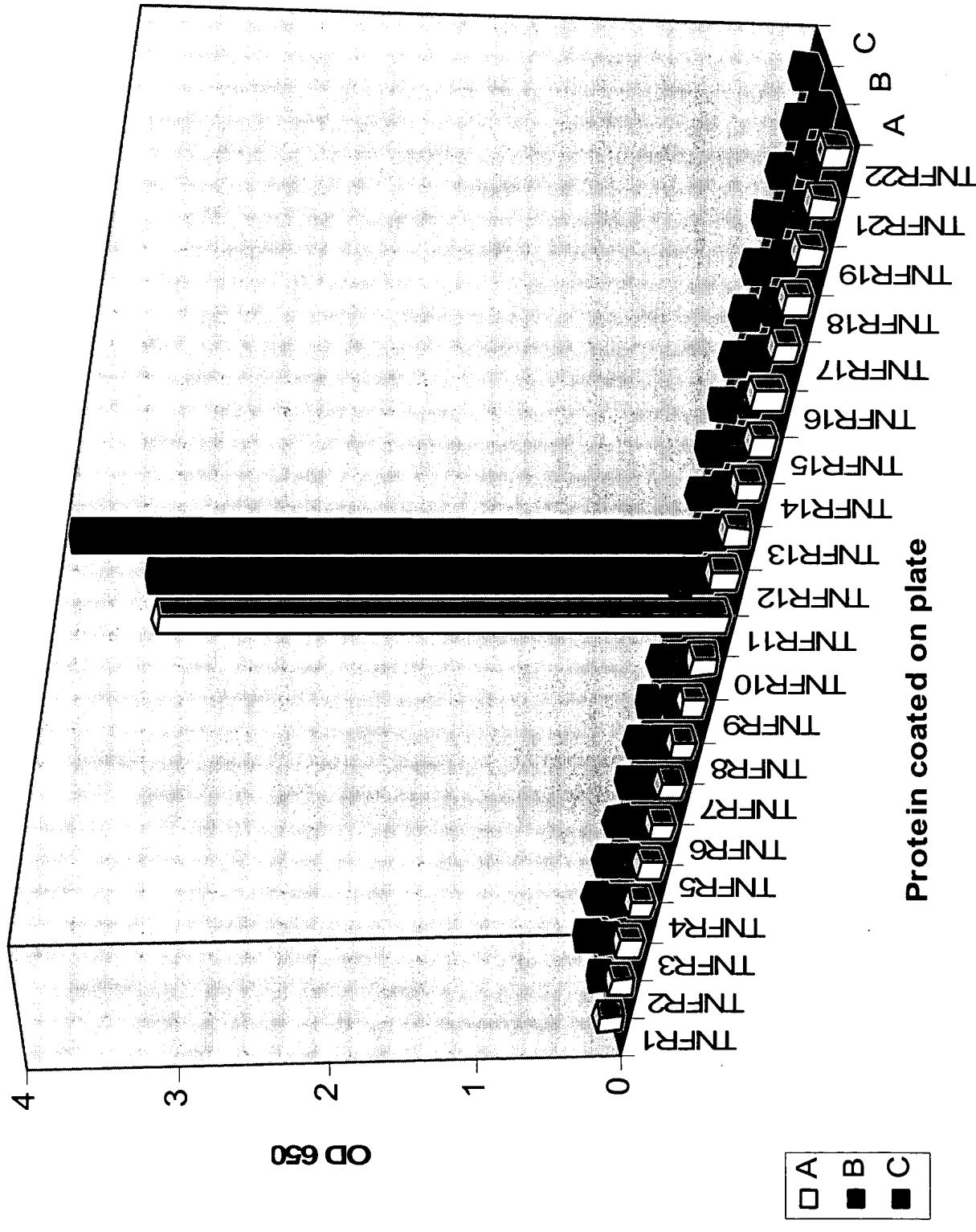


Figure 2

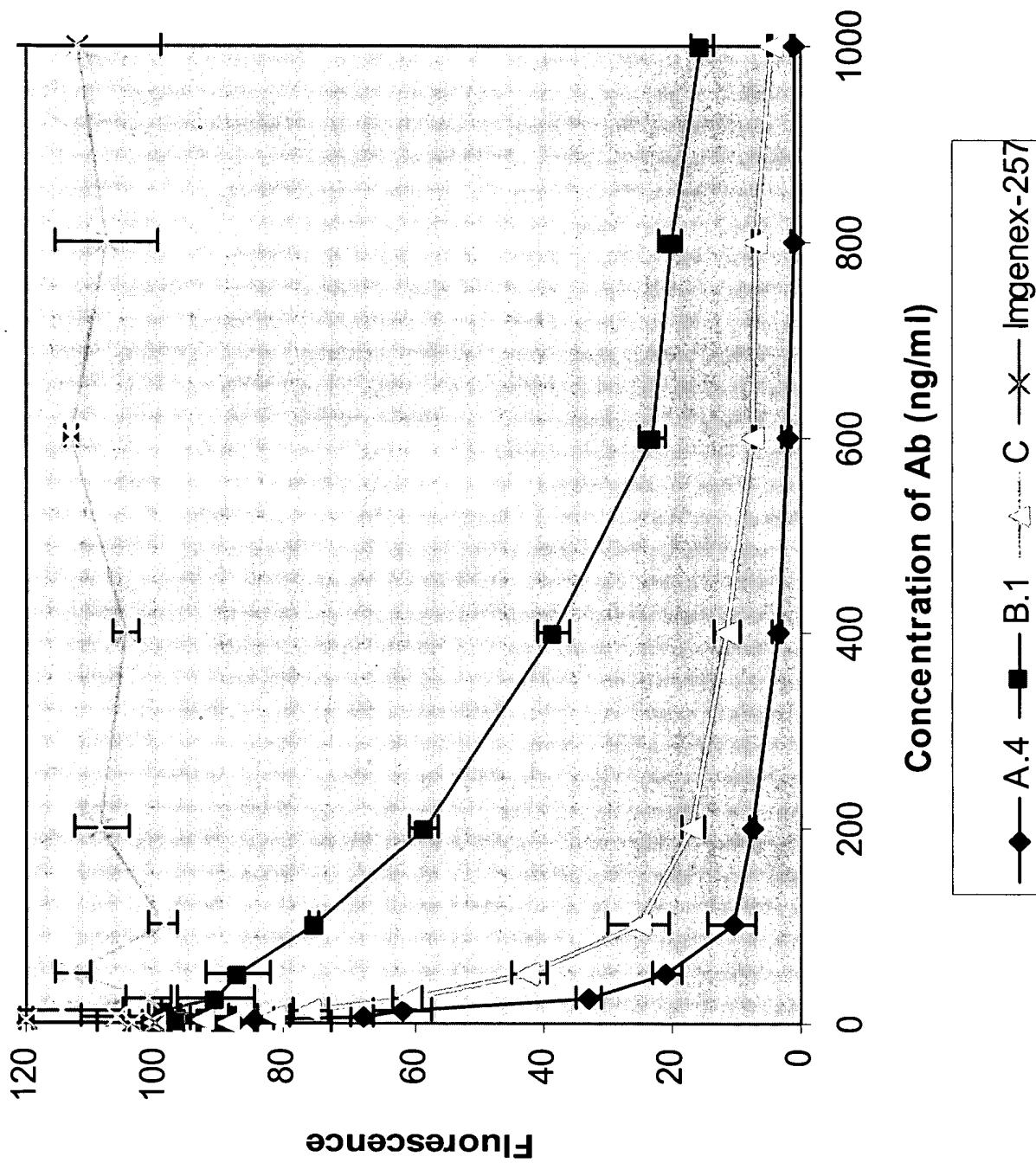
Specificity of DR5 Functional Antibodies



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Effect of 3 Different DR5 Antibody Agonists On Jurkat Cells

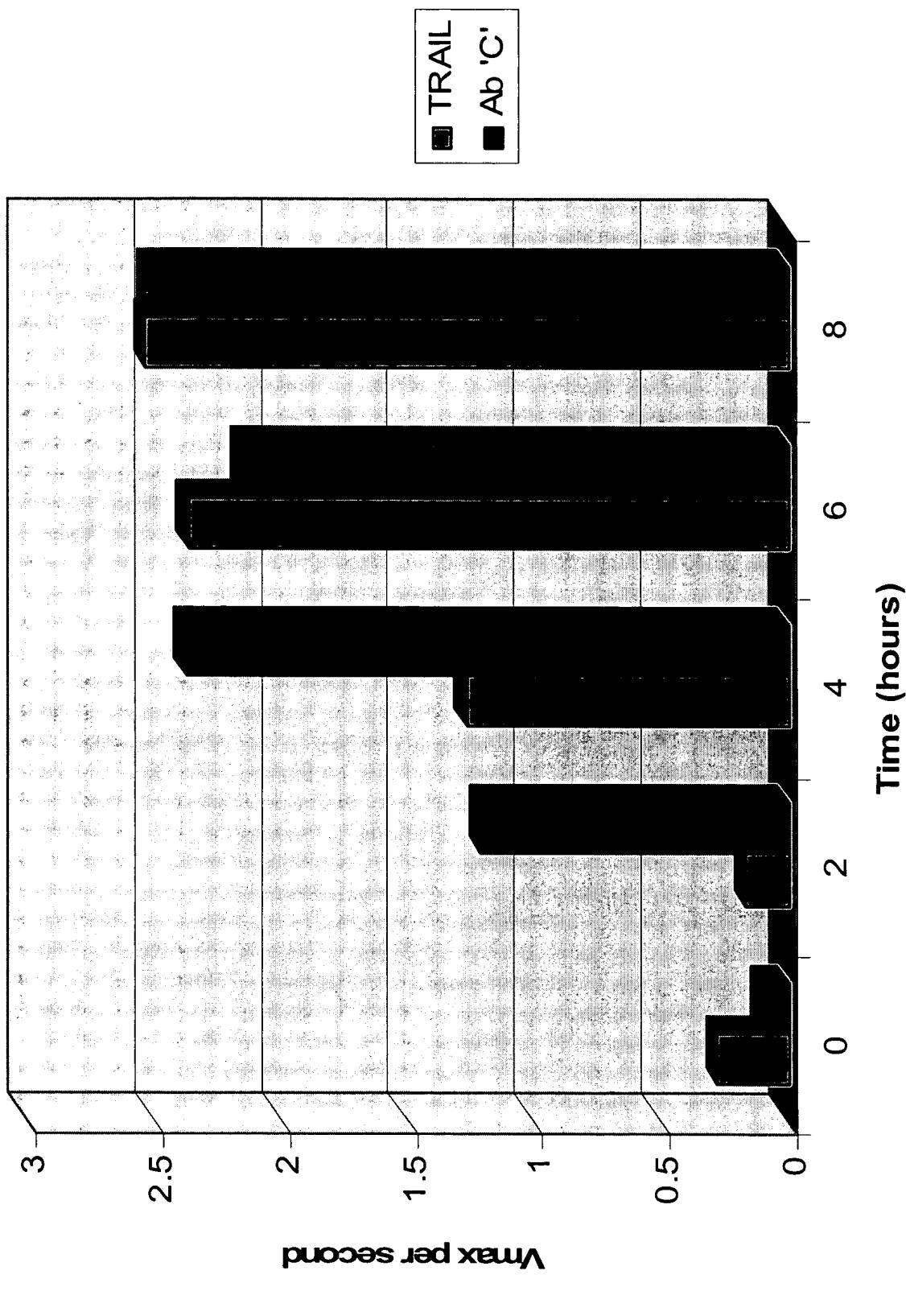
Figure 3



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Figure 4

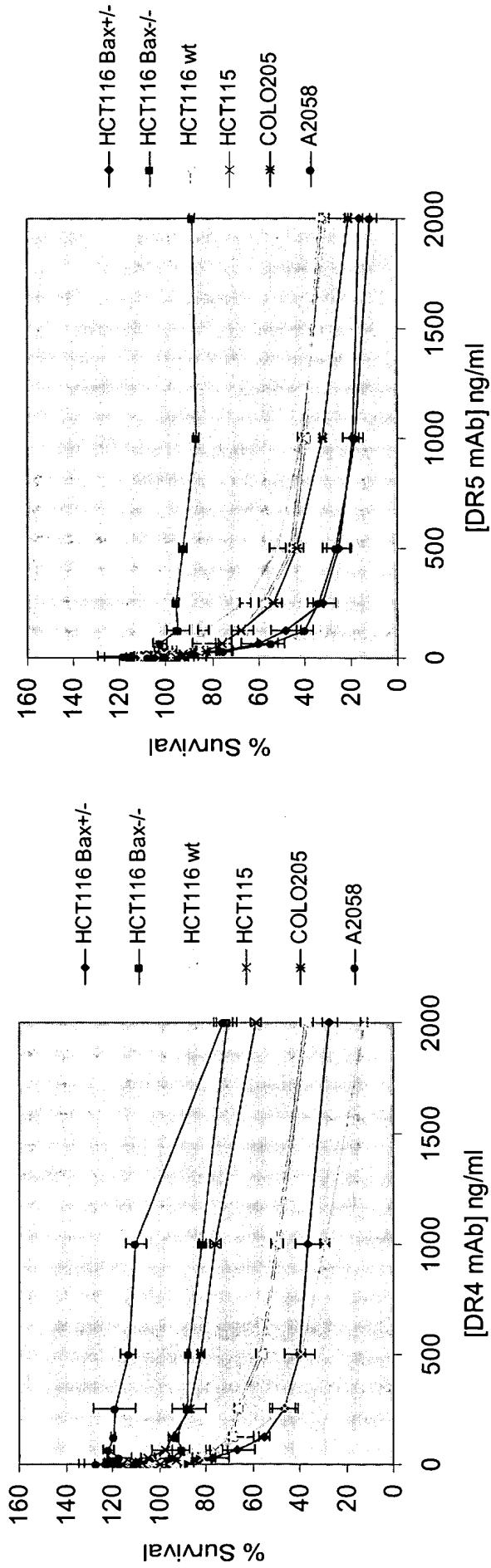
Caspase 3 activation in treated Jurkat cells



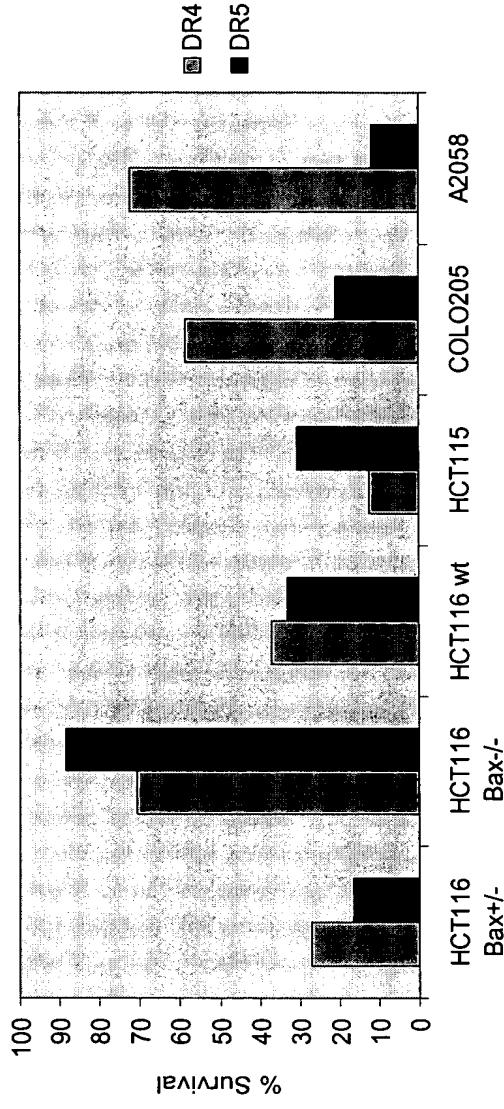
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Figure 5

Effect of DR4 / DR5 Functional Antibodies on Colon and Melanoma Cancer Cell Lines



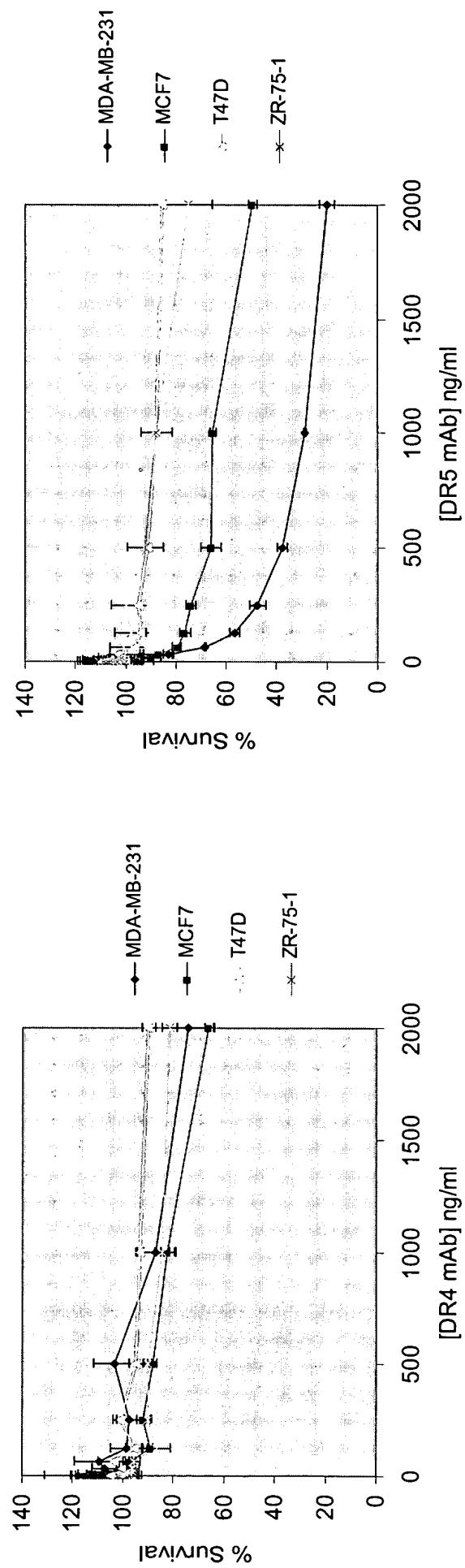
Effect of 2ug/ml Antibody on Cell lines



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Figure 6

Effect of DR4 / DR5 Functional Antibodies on Breast Cancer Cell Lines



Effect of 2ug/ml Antibody on various cell lines

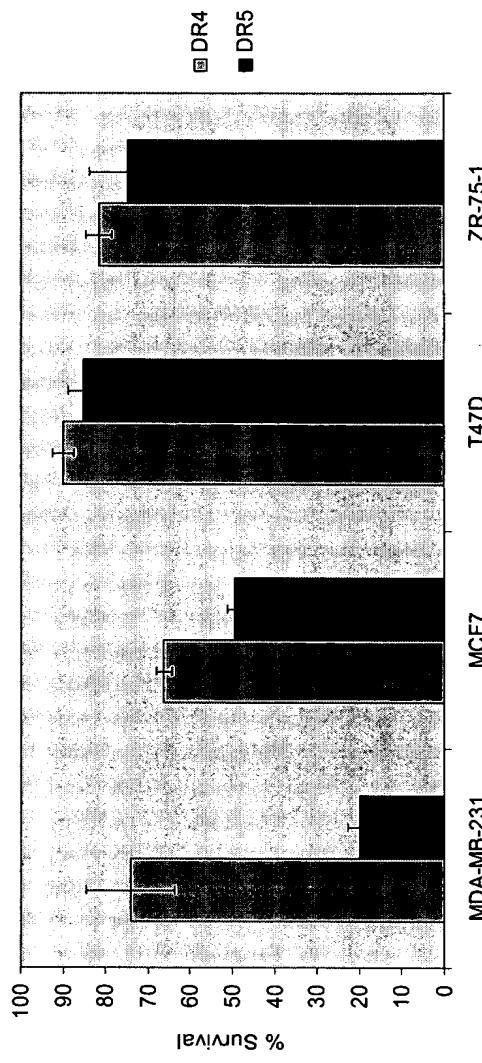


Figure 7

Dose Response To DR5 Antibody Agonist
Normal vs Tumor Cells

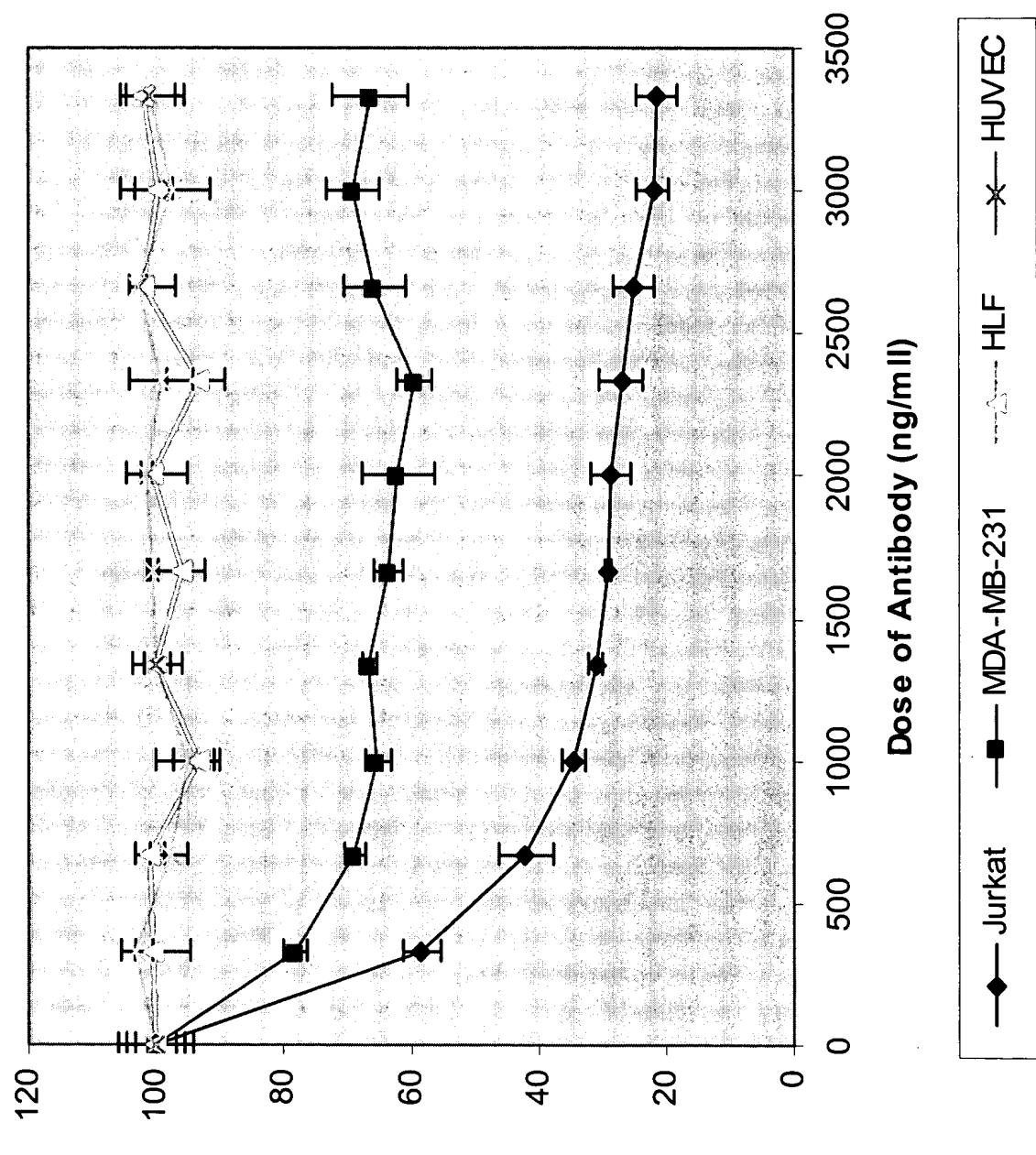


Figure 8

DR5 Antibody Agonist "A" - Caspase 3 Activation

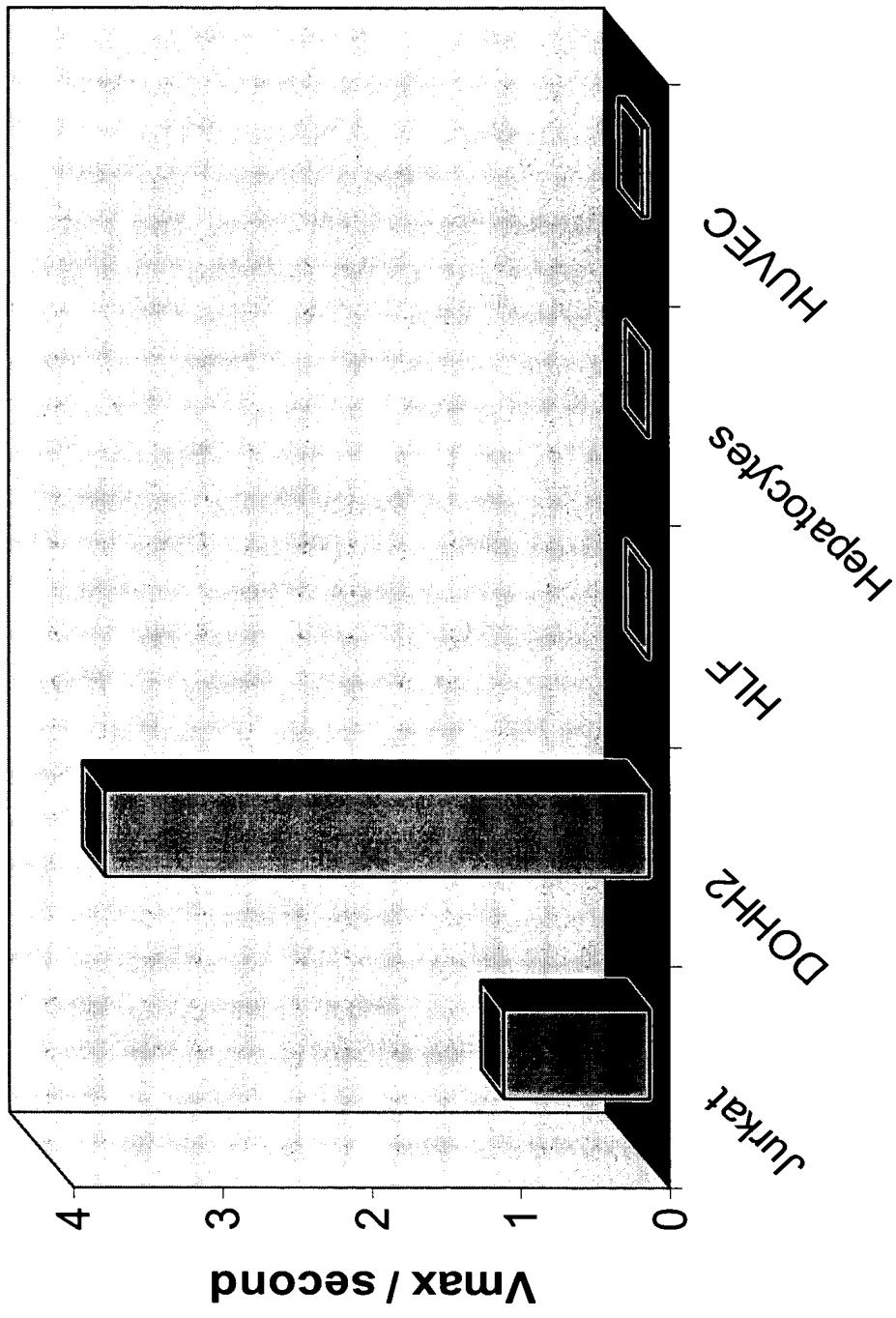


Figure 9

DR5 Antibody Agonist - Colo 205 Efficacy Study

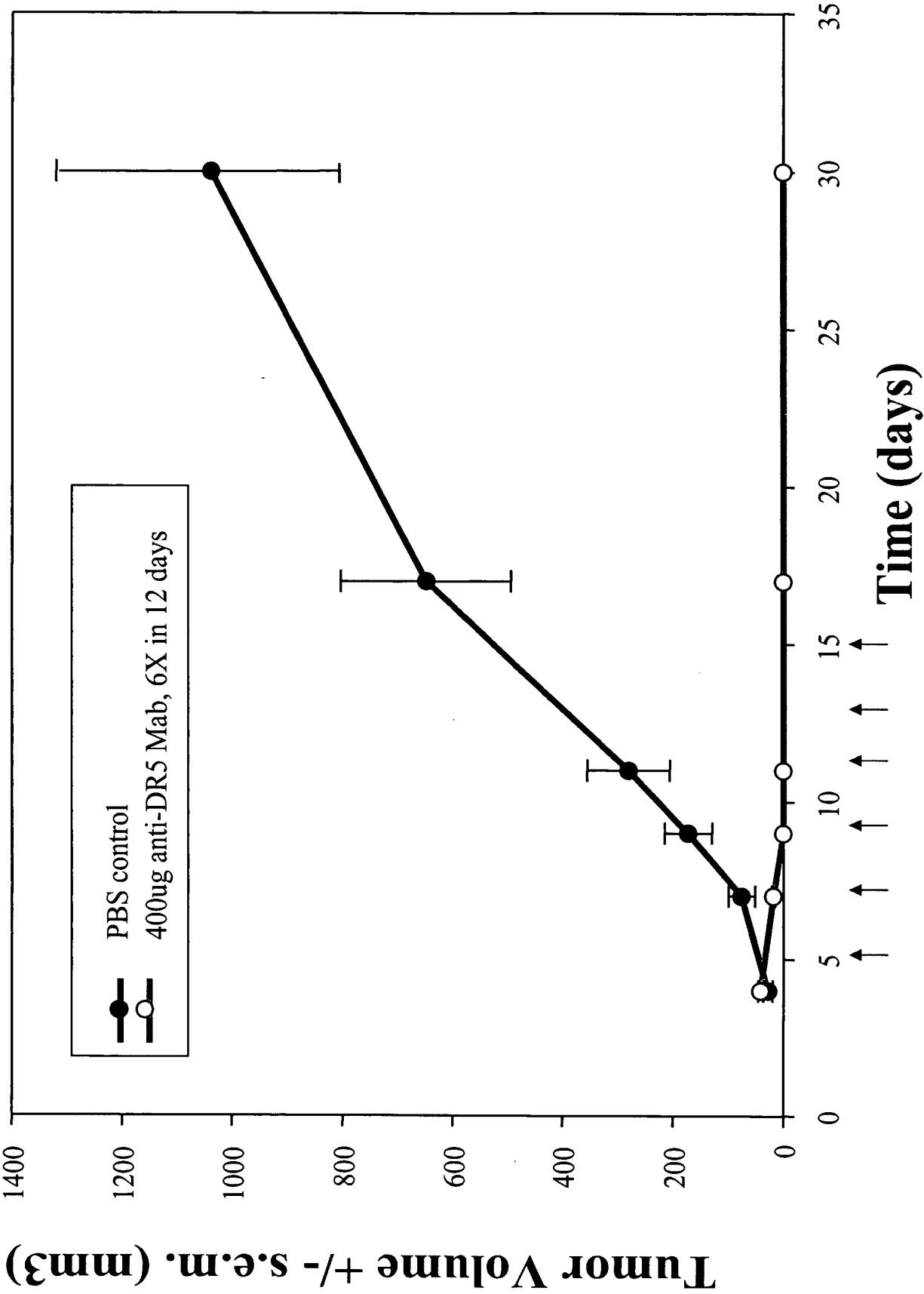


Figure 10

Anti-DR5 Dose Response, COLO205 Subcutaneous Model

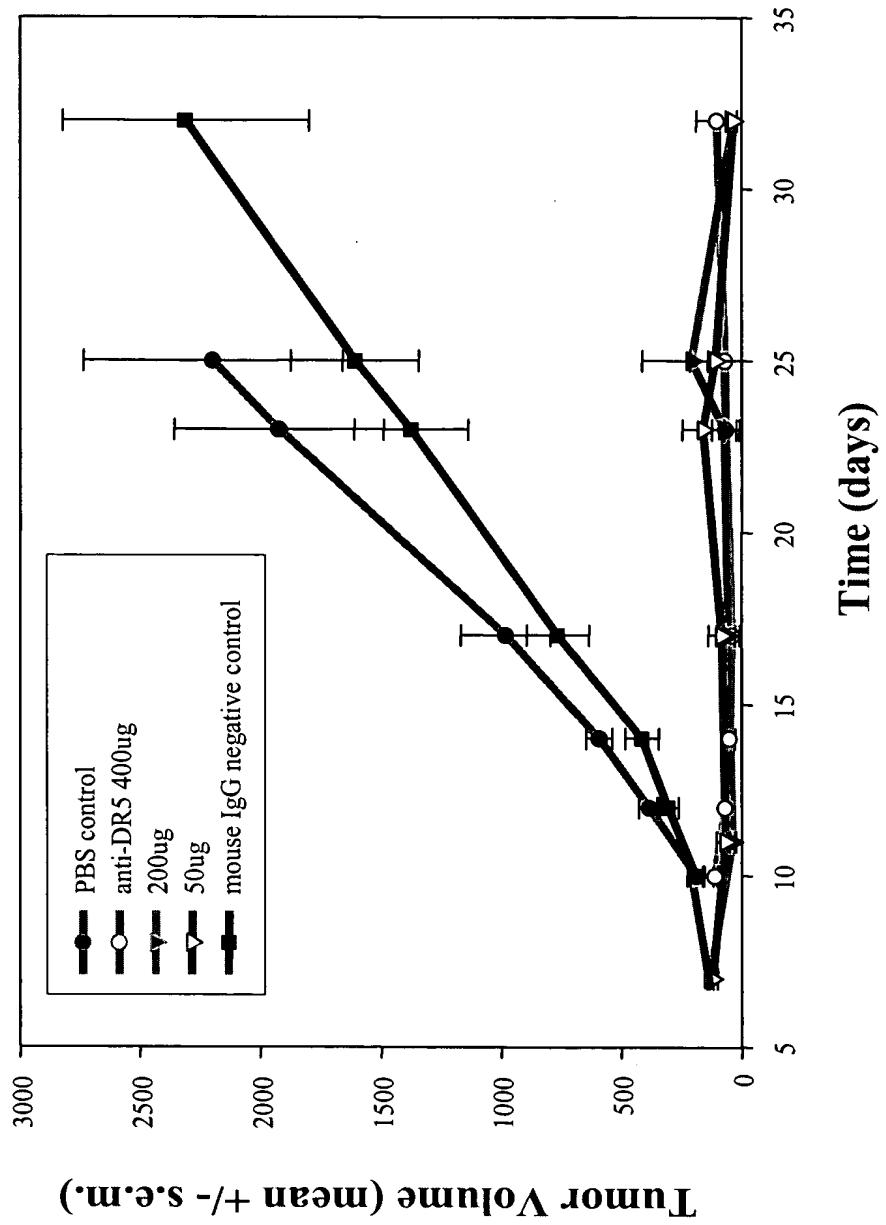
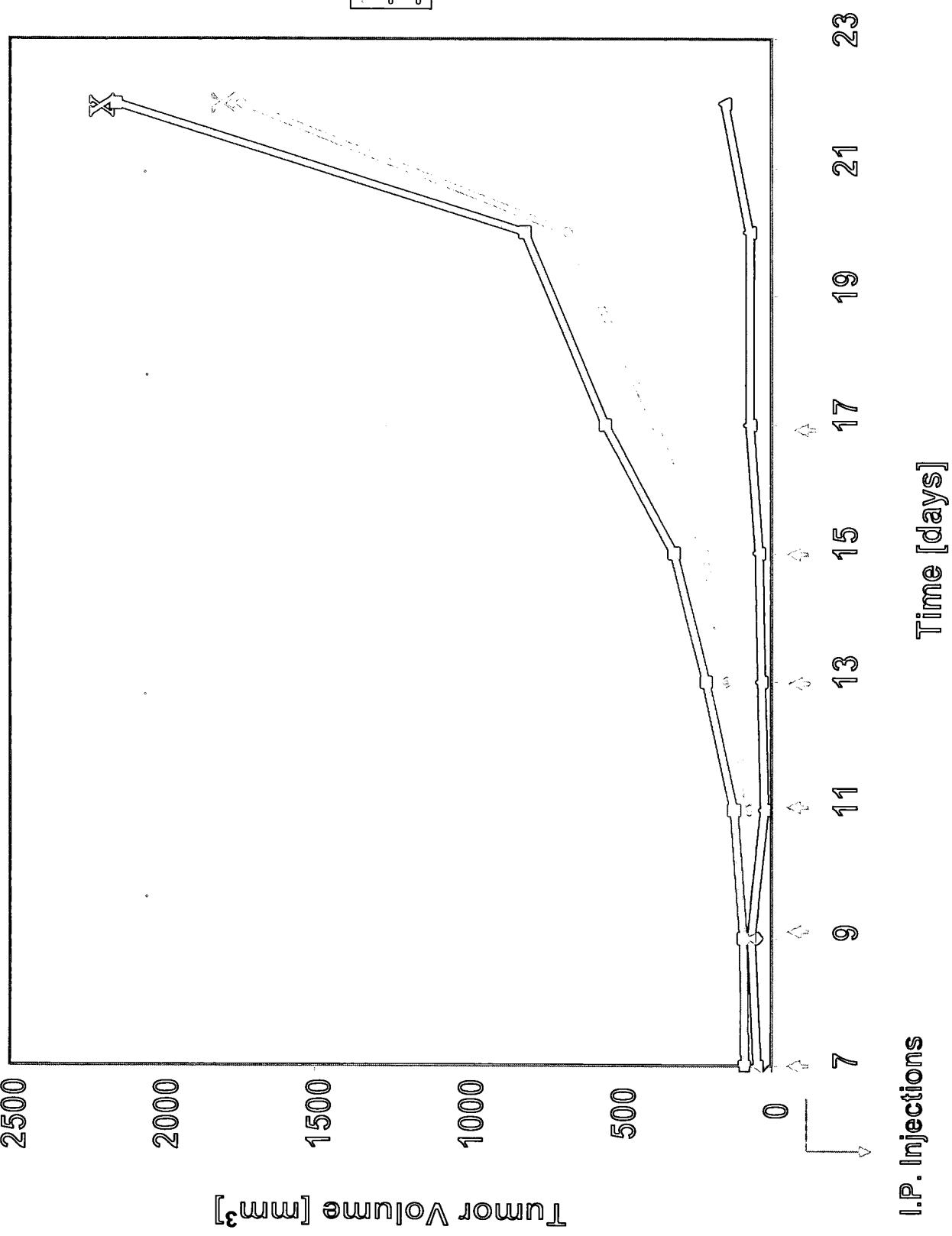


Figure 11
Tumoricidal Activity of DR5 Monoclonal Antibody In Vivo
A2058 Melanoma Model



Pathways For Caspase Activation and Apoptosis

Figure 12

TRAIL-Rs
Fas/CD95

EXTRINSIC

INTRINSIC

FADD
Pro-Caspase-8

Bid

t-Bid

Bak

Bax

Caspase-8

Apaf-1

Cyto-C

Mitochondria

Caspase-9

IAPs

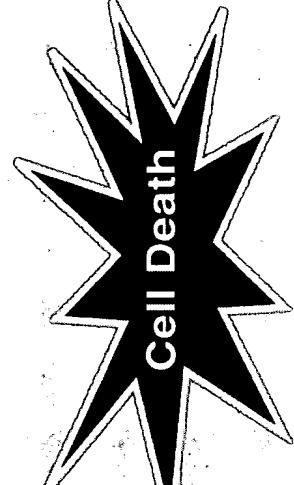
Caspase -3

Smac/
DIABLO

Bcl -2

Caspase

Death substrates



Predominant Anti-Apoptotic Features of Tumor Cells:

➤ Over-expression of Bcl 2

➤ Increased Levels of IAP's

➤ Mutations in Bax

Anti-DR4 or DR5-induced apoptosis in A2058 cells in the absence or presence of 0.5 uM LBP 672

Figure 13

24hr

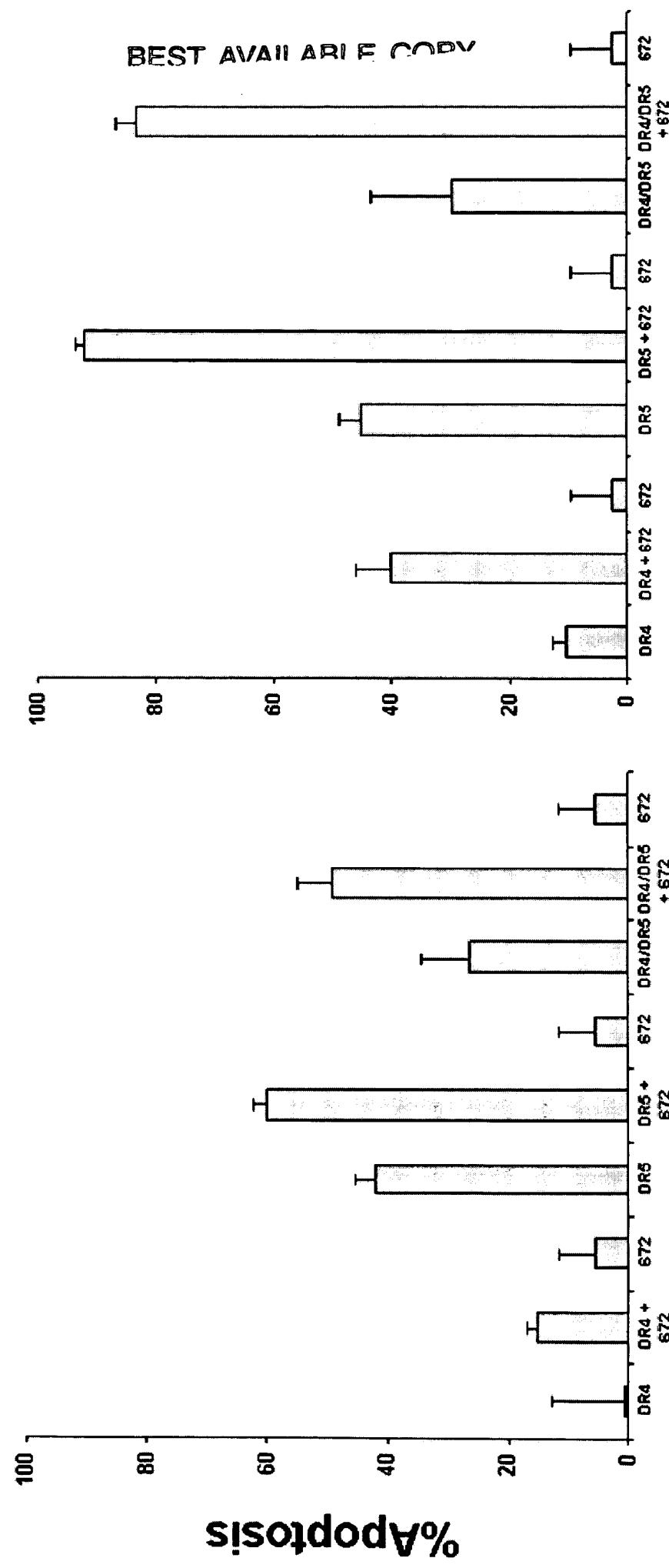


Figure 14

Effect of LB 672 On Normal And Tumor Cells

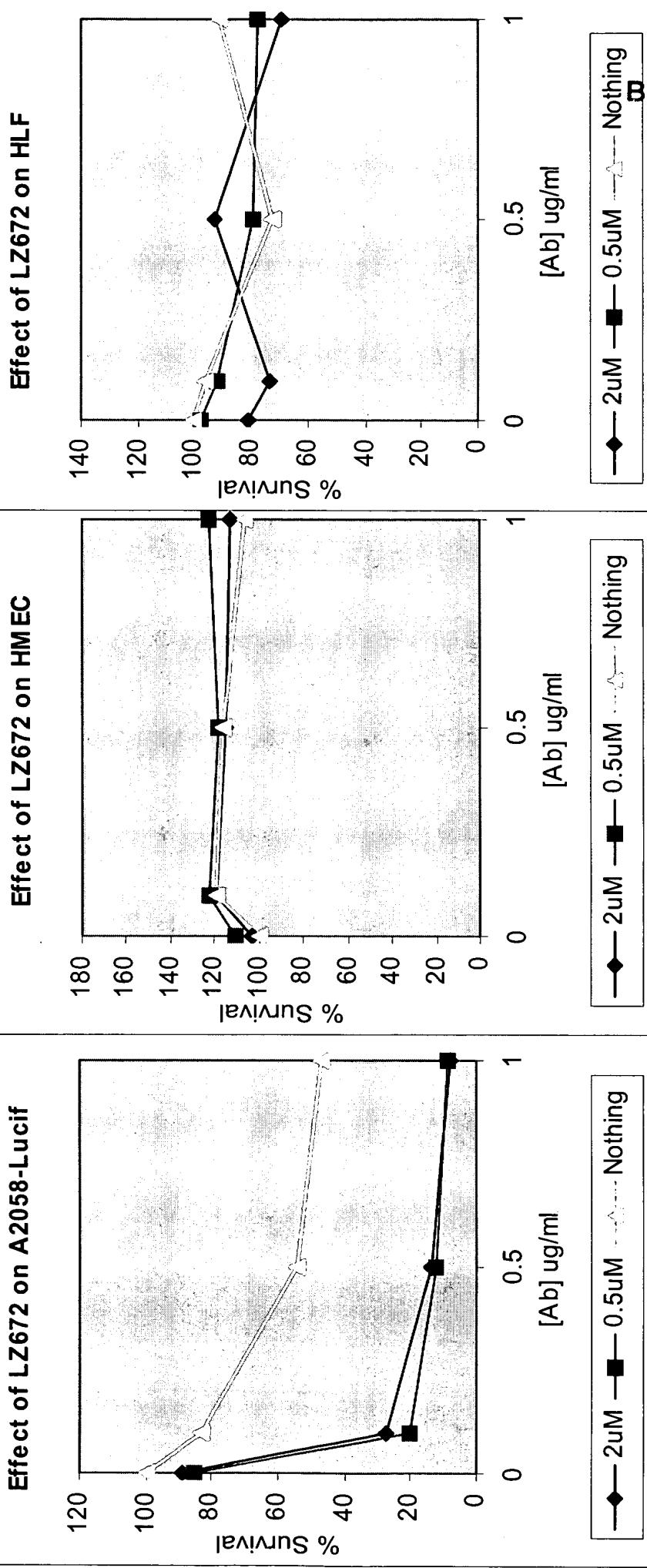
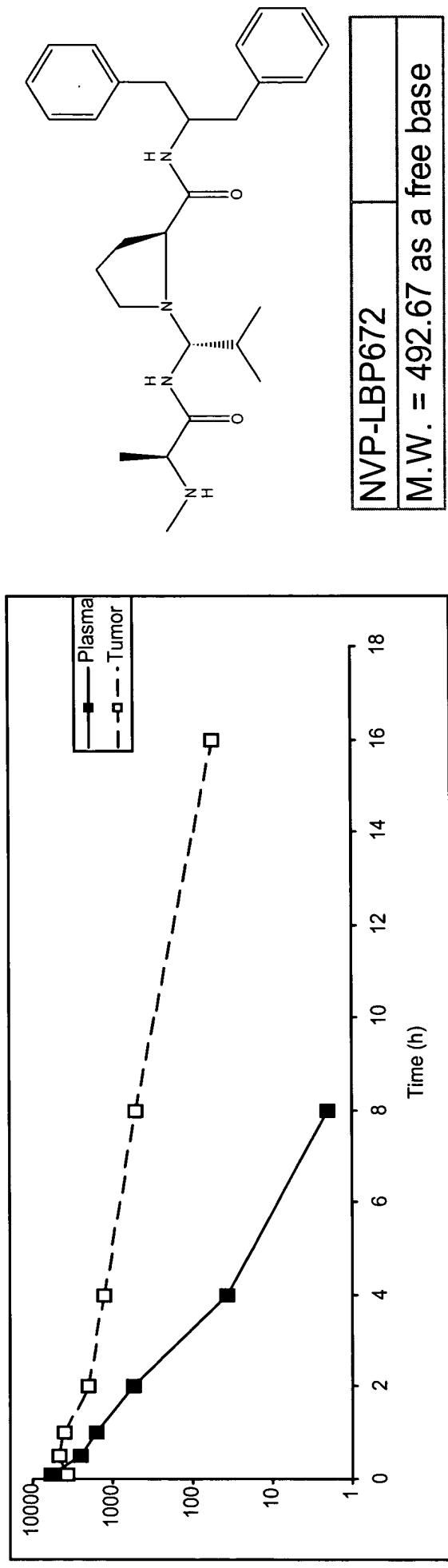


Figure 15

PK and PD Study of Smac Mimetic LBP672 In Nude Mice Bearing HCT116 Tumors



PK SUMMARY

Model: Athymic nude mice bearing subcutaneous HCT116 tumors

Dose: single 25 mg/kg i.v. trifluoroacetate salt (20.3 mg/kg free base) in D5W.

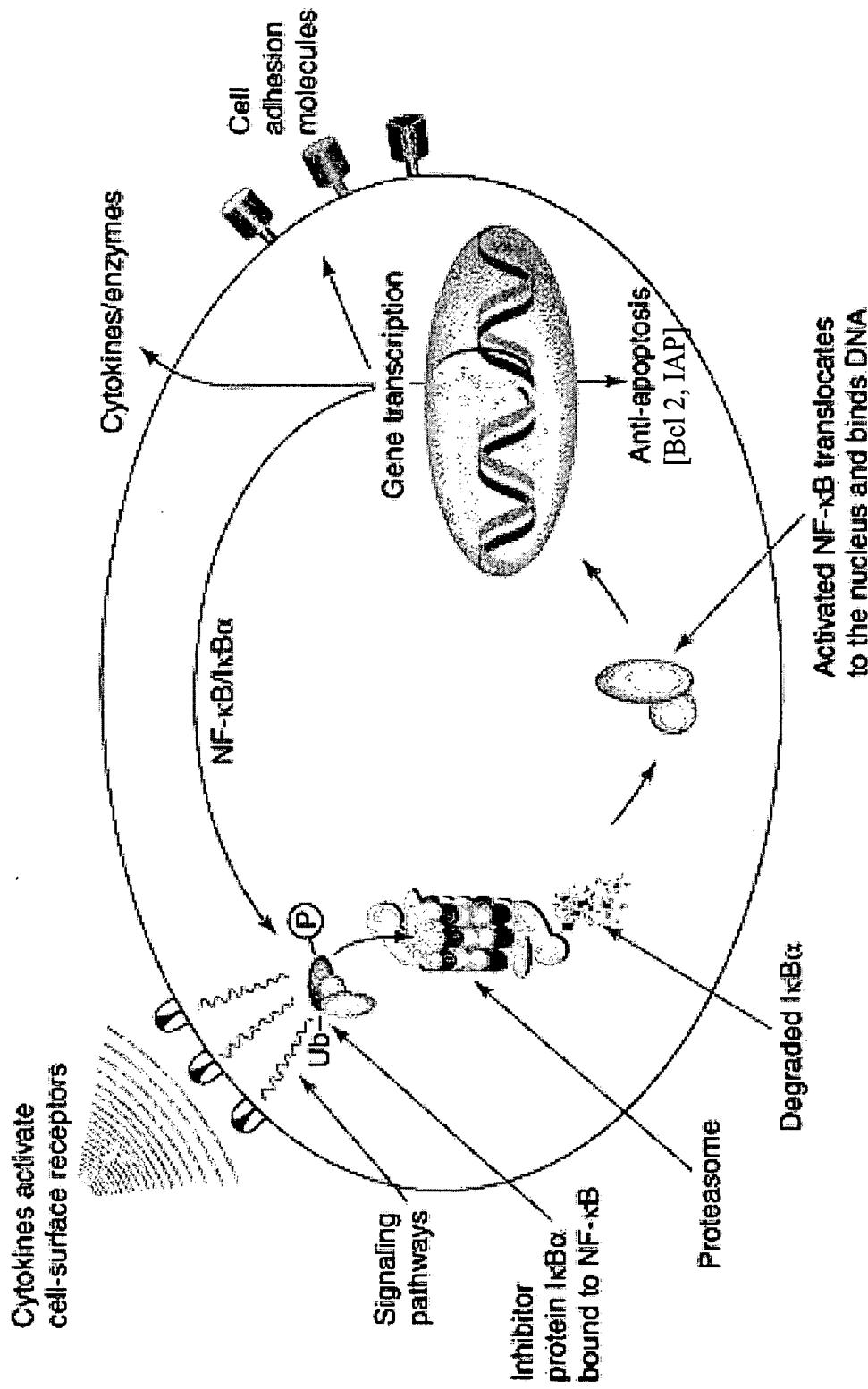
LBP672 rapidly absorbed by tumor Tmax @ 0.5 h. Mean tumor Cmax @ 4620ng/g (9.38 uM).

At 16 h. post dose LBP672 mean tumor [co] @ 55.1 ng/g (110 nM).

Figure 16

NF_κB Activation By The Proteosome

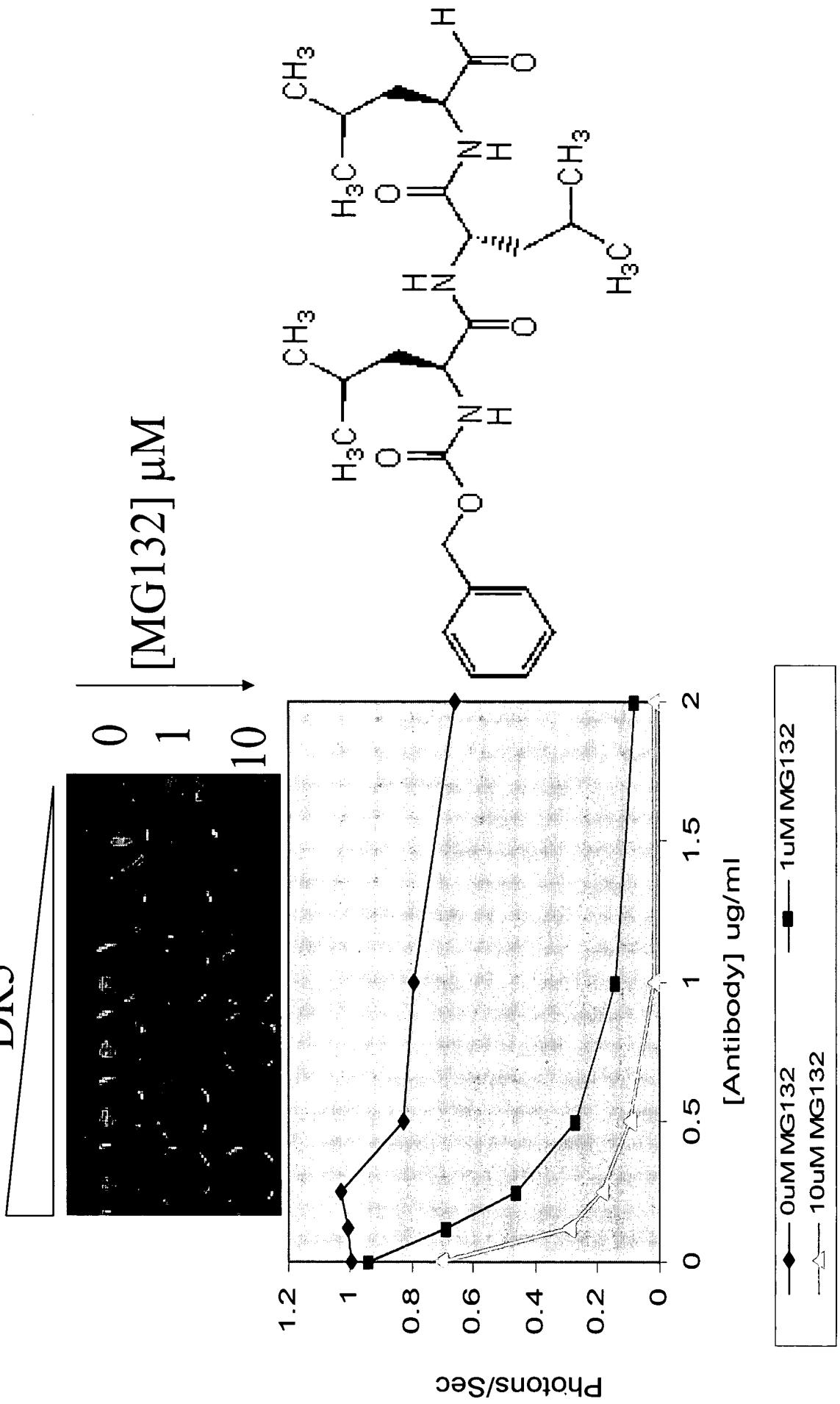
Viruses, growth factors, radiation or chemotherapeutic drugs activate pathways that lead to the degradation of I_κB by the proteosome. NF_κB activates transcription of genes that protect the cell from apoptosis



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Figure 17

Proteosome Inhibitor MG132 Enhances DR5 Antibody Induced Apoptosis of SW 480 Human Colon Carcinoma Cells DR5



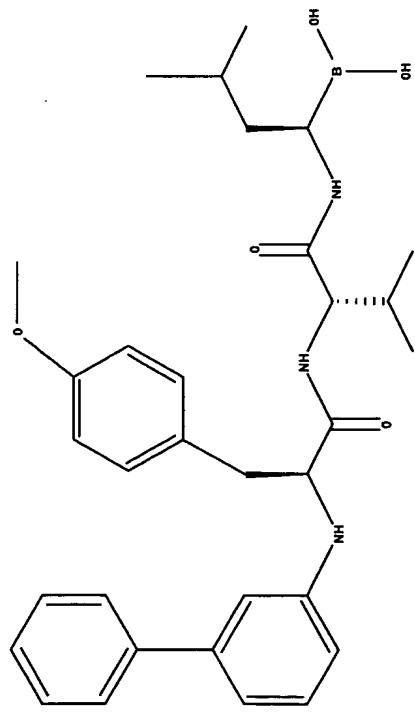
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Figure 18

20S Proteasome Inhibition - [Chymotryptic Activity]

Compound	Description	Selectivity	IC50	MTD	Sol.
NVP-AFB340-NX	Boronate	Good	<1nM	3mg/kg-1x	Good
NVP-AFD314-NX	Boronate	Good	<1nM	3mg/kg-1x	Good
NVP-AEV273-NX	Beta-Lactame	Excellent	3nM	20 mg/kg-2x	Poor

NVP-AFB340-NX



Effect of Proteosome Inhibitors On A2058-Luc

Figure 19

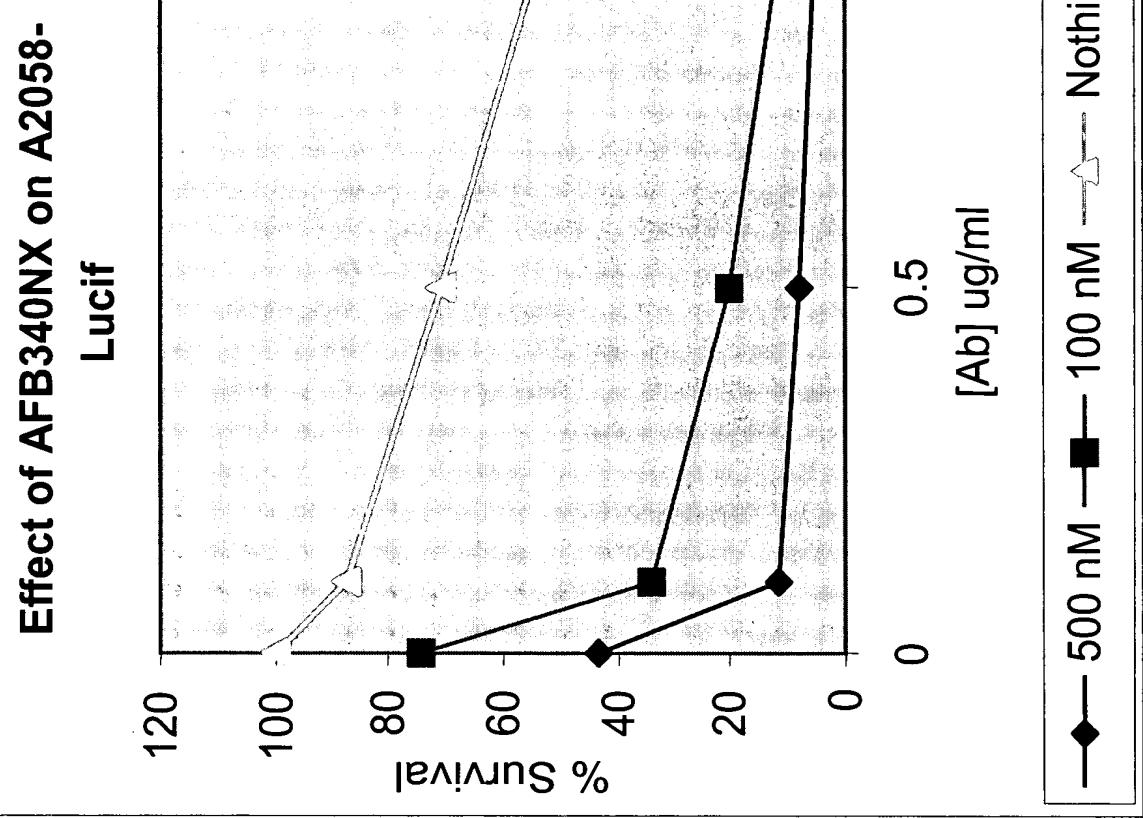
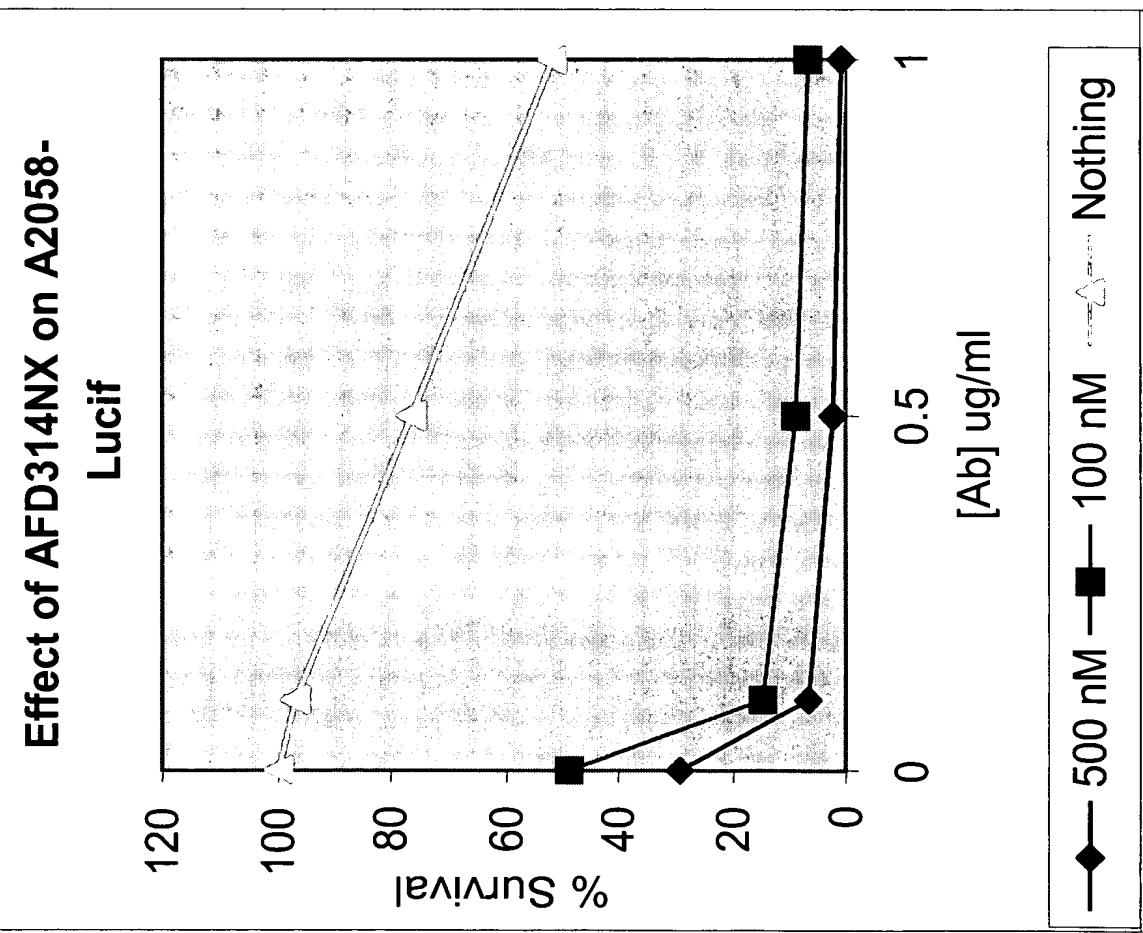
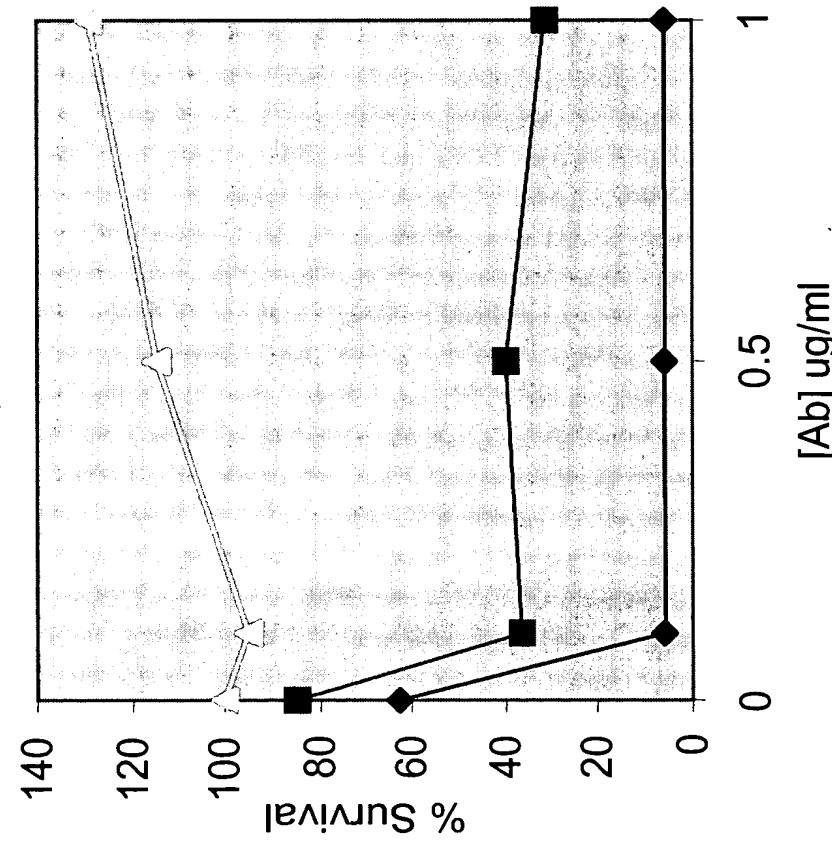


Figure 20

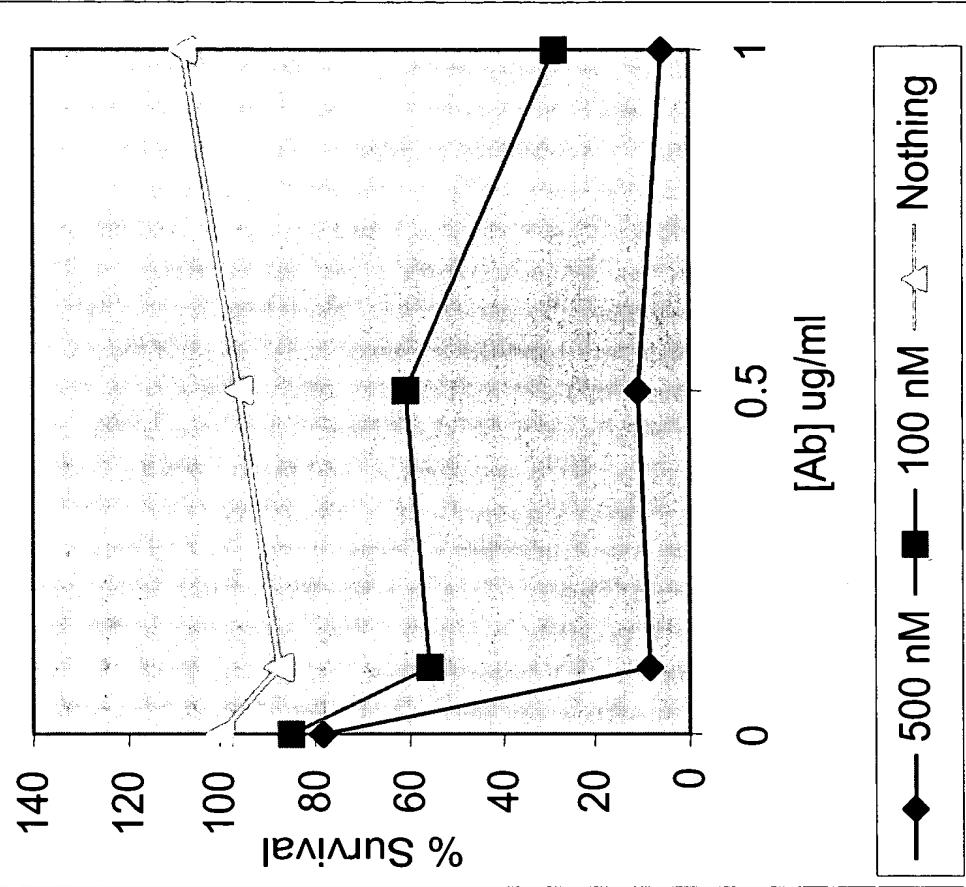
Effect of Proteosome Inhibitors On The Hepatocarcinoma

Cell Line HUH7-Luc

Effect of AFD314NX on HUH7-Lucif



Effect of AFB340NX on HUH7-Lucif



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Figure 21

Effect of Proteosome Inhibitors On Normal Human Mammary Epithelial Cells (HMEC)

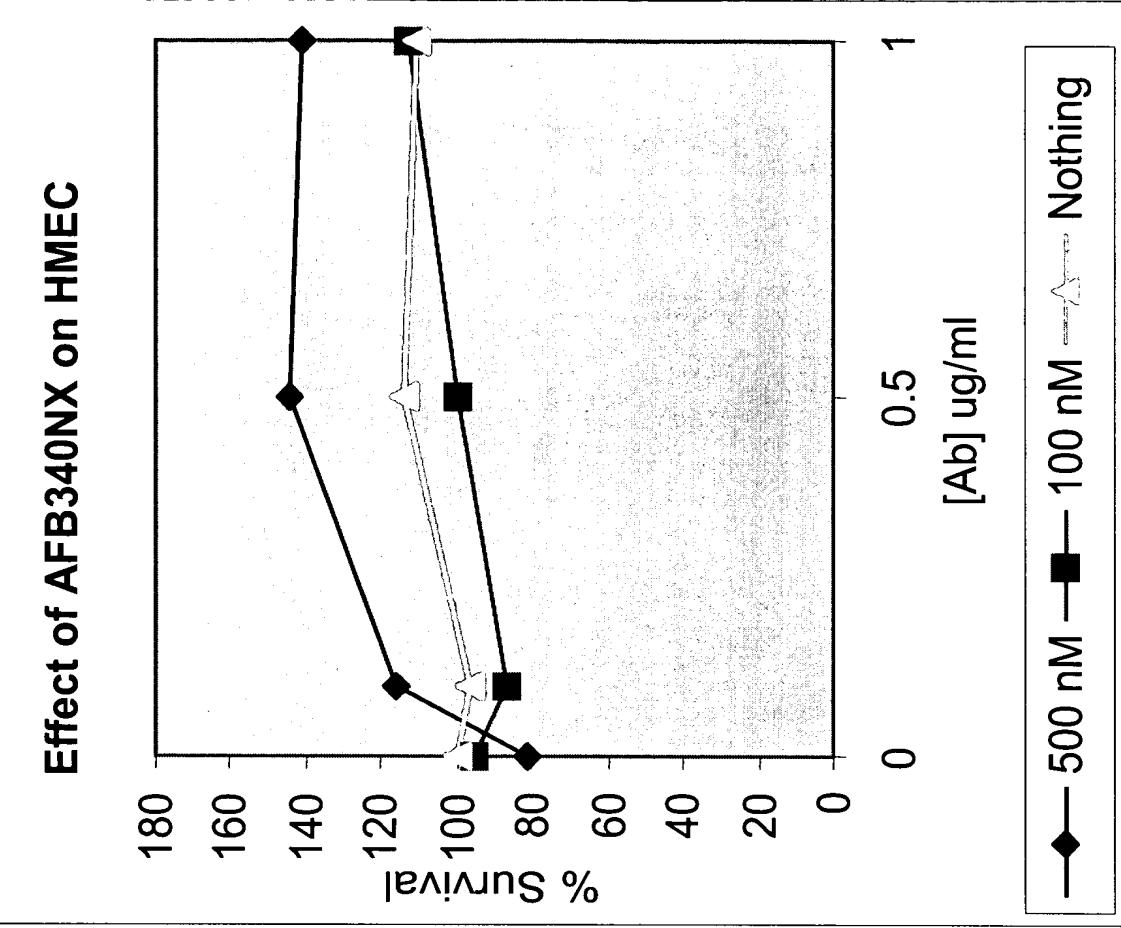
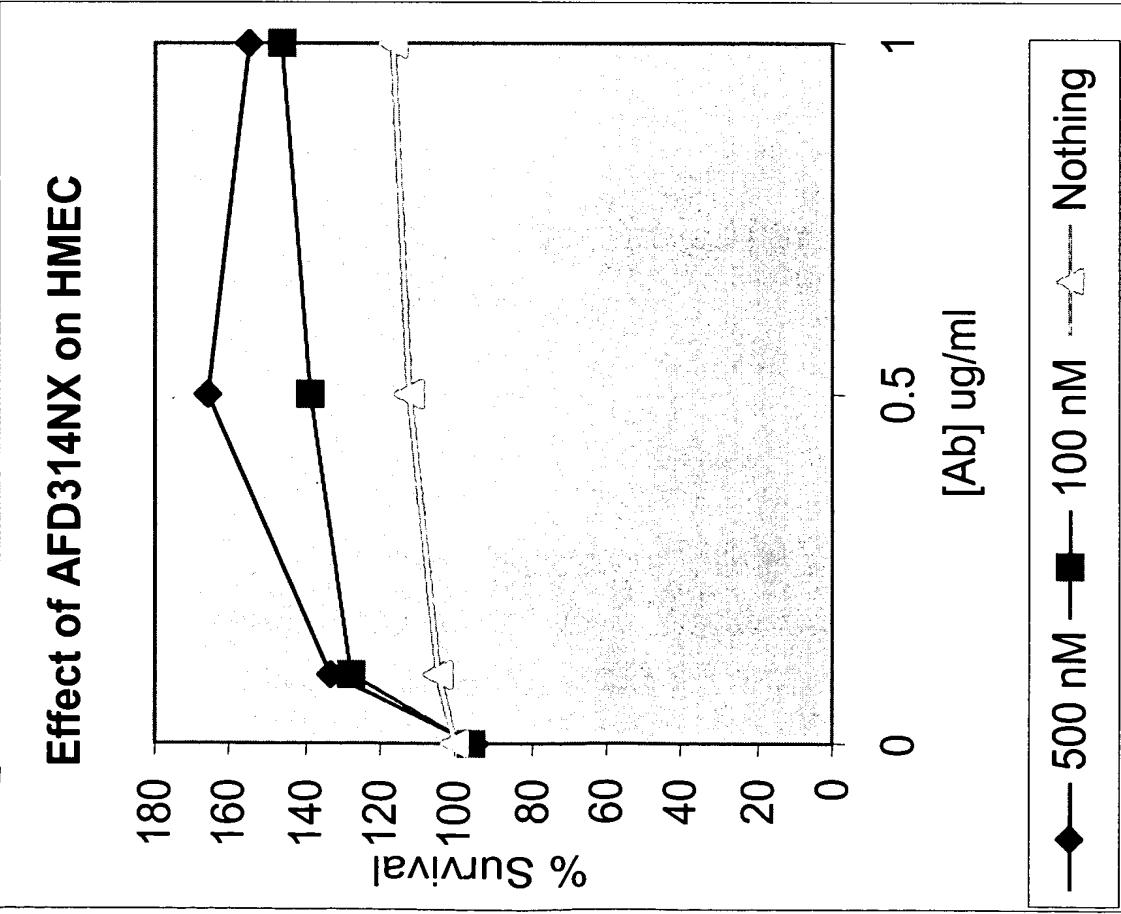


Figure 22

Functional Activity

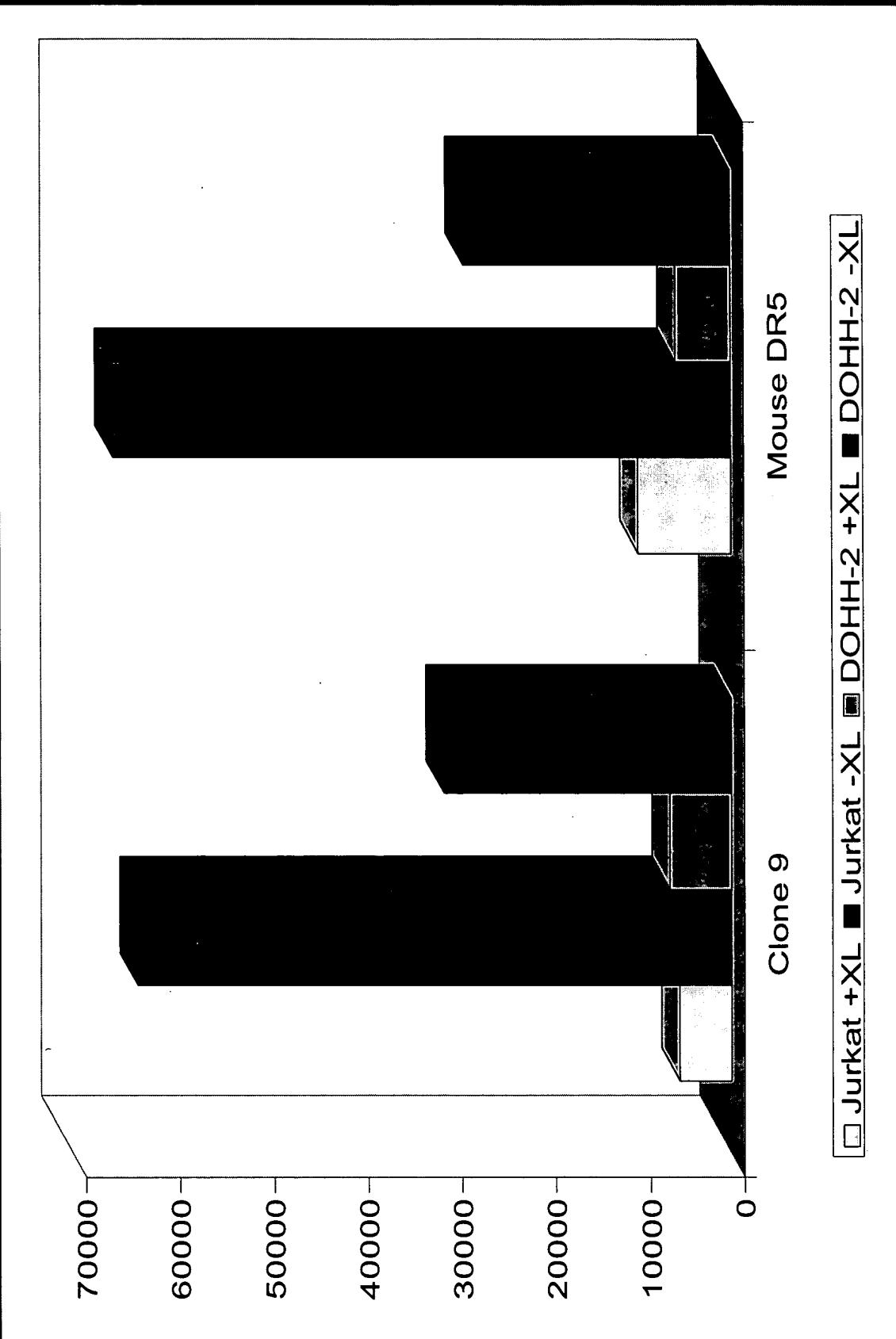


Figure 23

Anti-DR5 DNA Sequence

Light Chain Variable Region

GACATTGGCGATGACCCAGTCTCACAAAGTTCATGTCCACATTAGTGGGAGACAGGGTCA
GCATCACCTGCAAGGCCAGTCAGGATGTGAATACTGCTTACCTGGTATCAACAAAAA
ACCAGGGCAATCTCCTAAACTGATTACTGATCTGGCATCCACCGCACACTGGAGTC
CCTGATCGCTTCACAGGCAGTGGATCTGGACAGATTACTCTACCATCAGCAGTA
TGGAGGGCTGAAGAGATGCTGCCACTTATTACTGCCAGTGAGTAGTAAACCCGCTCAC
GTTGGGTGCTGGGACCAAGCTGGGCTGAAACGGGCTGATGCTGACCAACTGTATCC
ATCTTCCCCACC

Heavy Chain Variable Region

CAGGCCAAGGGTCCAGCAGCTGCAGGCAGTCTGGGAGGCTGGTGAACCCGGGCATC
GTGAAGGCTGTCCCTGCAAGGGCTCTGGCTACACCCCTCACTGACTATACTACACTGGGT
AAAGCAGGGTCTGGACAGGGCTTGAAGTGGGATTGGGTGTTACCCCTGGAGGGTGGGT
TATATAAAATACAATGAGAAATTCAAGGACAGGGCCACATTGACTGCGGACAAATCCT
CCAACACAGTCTATGGAGCTTACTGACATCTGAAGGGCTCTGGGTCTATTTC
TGTGCAAAGACACGAAGAGGGCATCTTTGACTACTGGGCCAAGGCACCAACTCTCA
CAGTCTCCTCA

Figure 24

DR5 V_H Sequence – Heavy Chain Subgroup 2B

FR1

Lys Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val

CDR1

Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Thr Ile His Trp Val

FR2

Lys Gln Arg Ser Gly Gln Gly Leu Glu Trp Ile Gly Trp Phe Tyr Pro Gly Gly

CDR2

Gly Tyr Ile Lys Tyr Asn Glu Lys Phe Lys Asp Arg Ala Thr Leu Thr Ala Asp

FR3

Lys Ser Ser Asn Thr Val Tyr Met Glu Leu Ser Arg Leu Thr Ser Glu Gly Ser

CDR3

Ala Val Tyr Phe Cys Ala Arg His Glu Glu Gly Ile Tyr Phe Asp Tyr Trp Gly

FR4

Gln Gly Thr Thr Leu Thr Val Ser Ser

Figure 25

DR5 V_L Sequence–Kappa Light Chain Subgroup 5

FR1
Asp Ile Ala Met Thr Gln Ser His Lys Phe Met Ser Thr Leu Val Gly Asp

CDR1
Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala Ile Ala

FR2
Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Ile Tyr Trp Ala

CDR2
Ser Thr Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly

FR3
Thr Asp Tyr Thr Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala Thr

CDR3
Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Leu Thr Phe Gly Ala Gly Thr

FR4
Lys Leu Glu Leu Lys Arg Ala

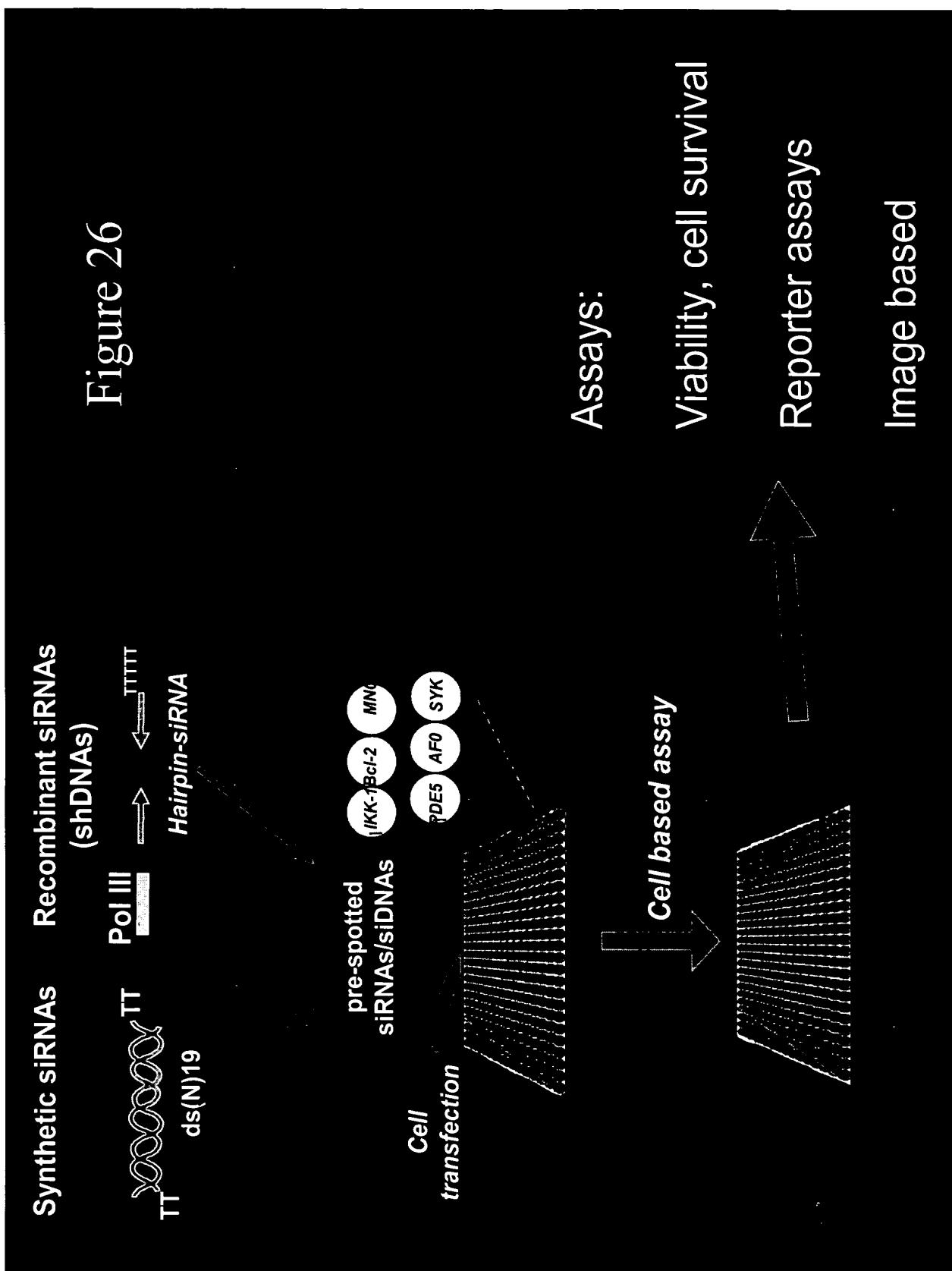
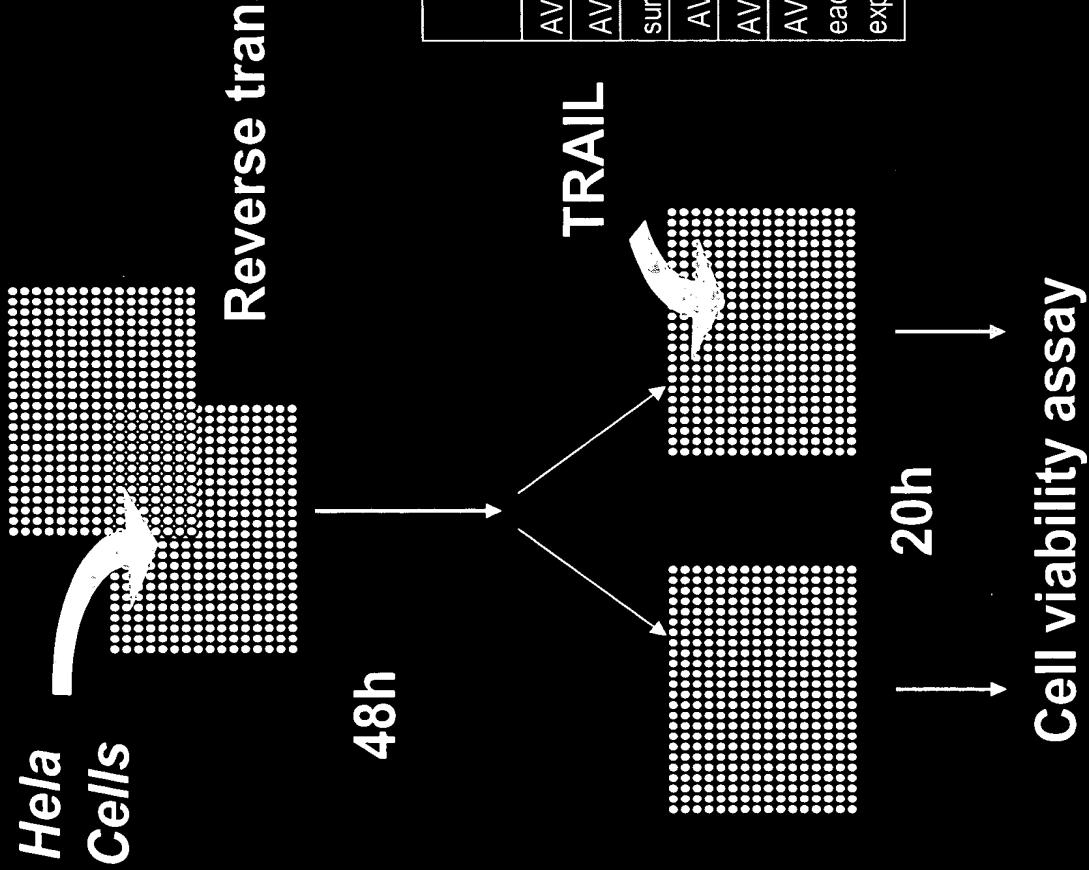


Figure 27

TRAIL induced apoptosis in HeLa cells



	screen 1		screen2	
	(-TRAIL)	(+TRAIL)	(-TRAIL)	(+TRAIL)
AV row data	29221.7	9368.75	59419.2	26782.7
AV normalized controls	100	32	100	45
survival ratio controls	0.32		0.45	
AV SD normalized controls	7.06	16.12	6.18	10.76
AV SD among duplicates	5.75	8.02	4.11	6.71
AV SD of survival ratio for each siRNA among the 2 experiments			12.87	

Screen

Figure 28

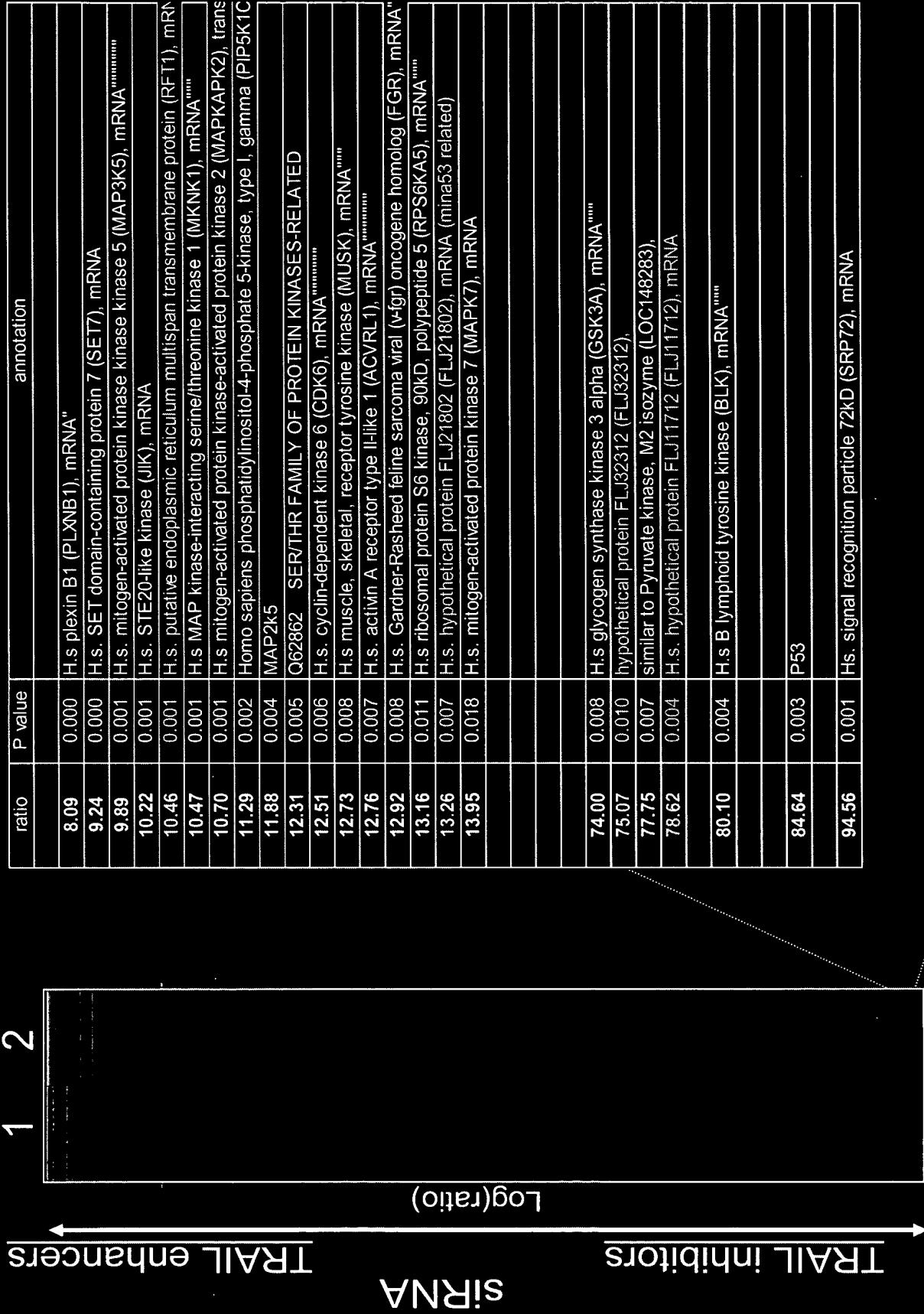


Figure 29

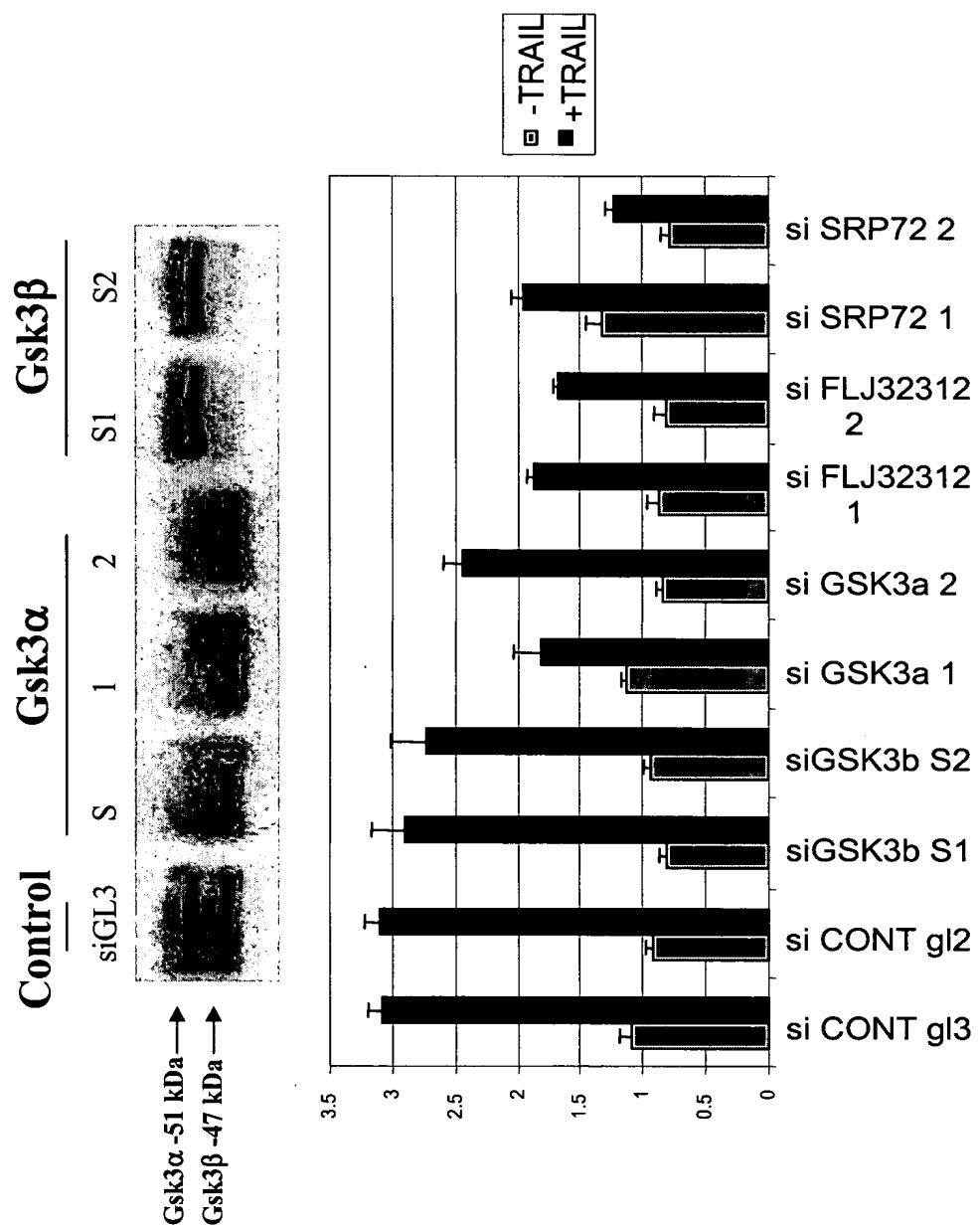


Figure 30

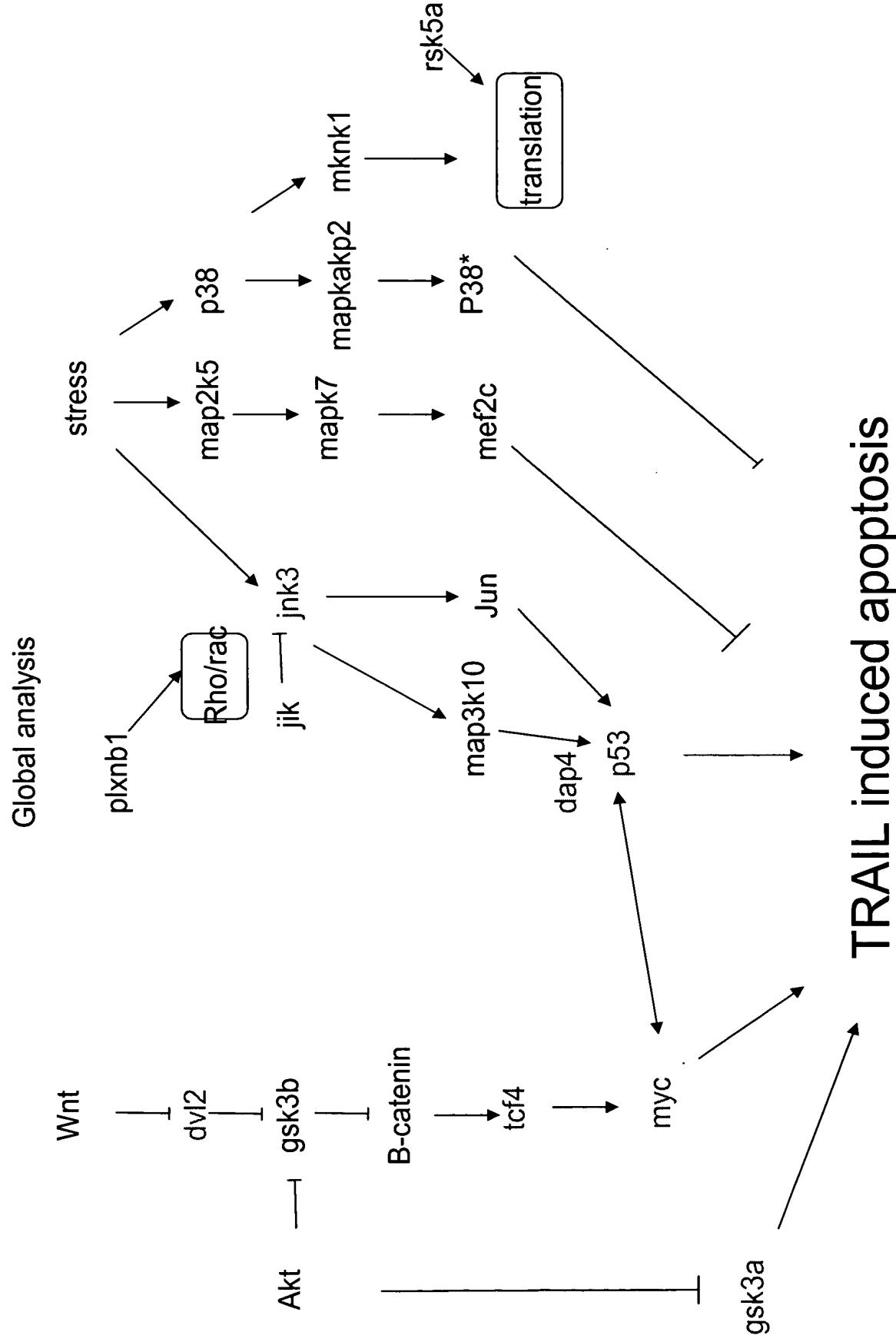
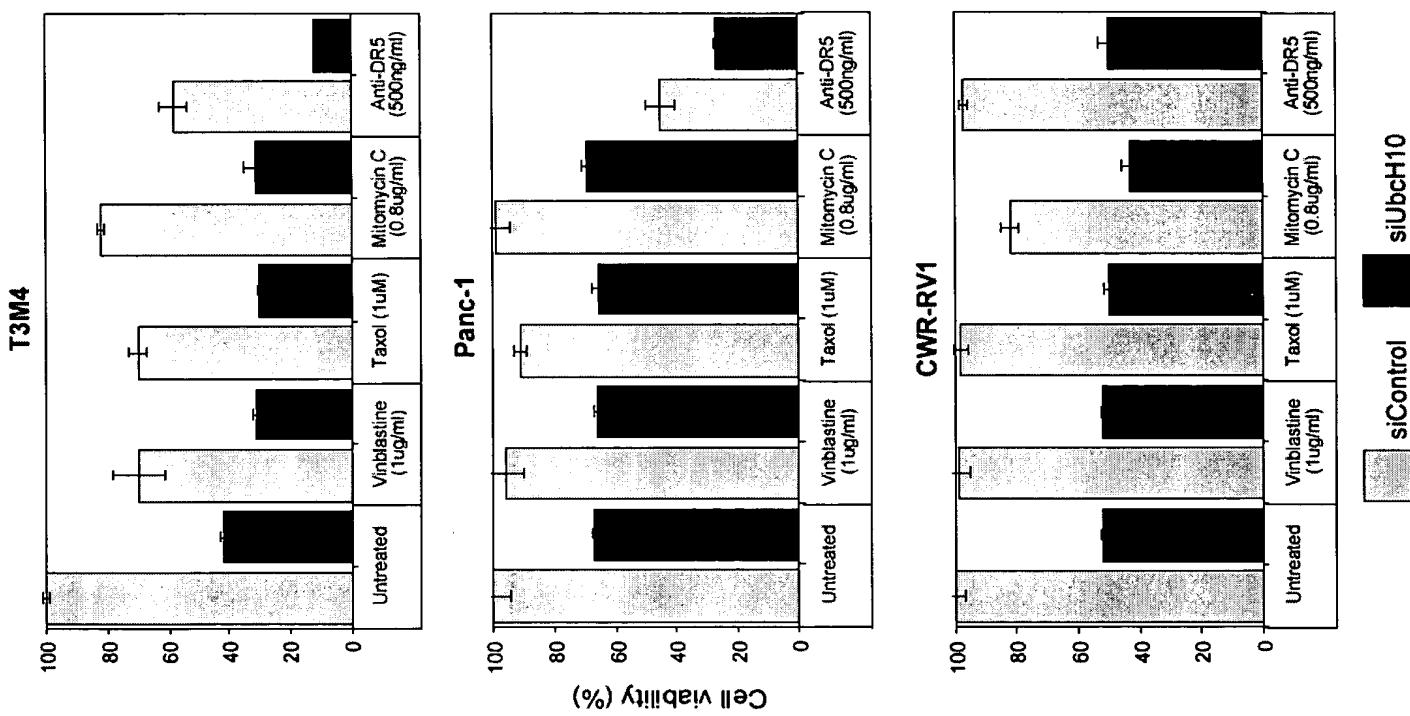


Figure 31



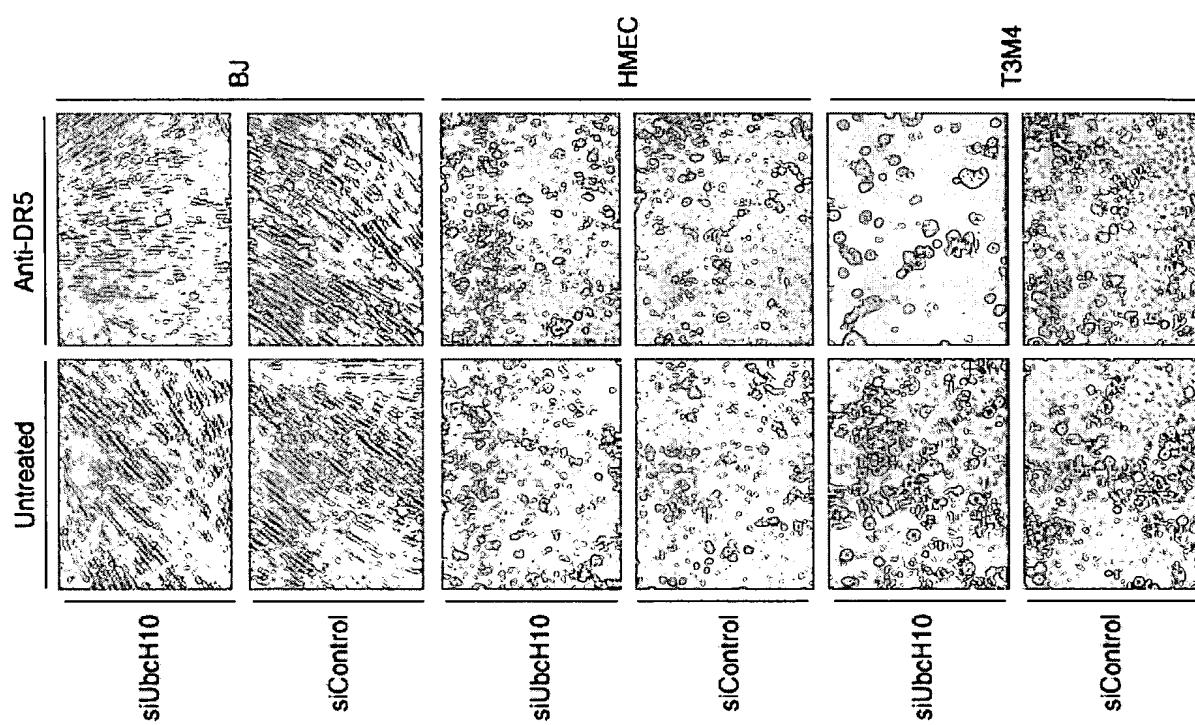
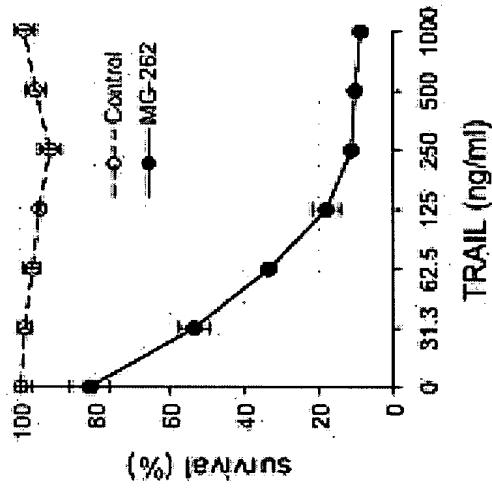
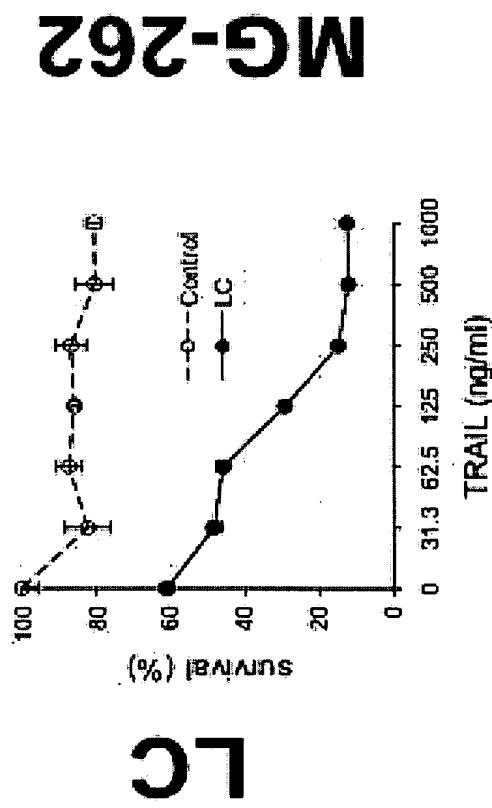
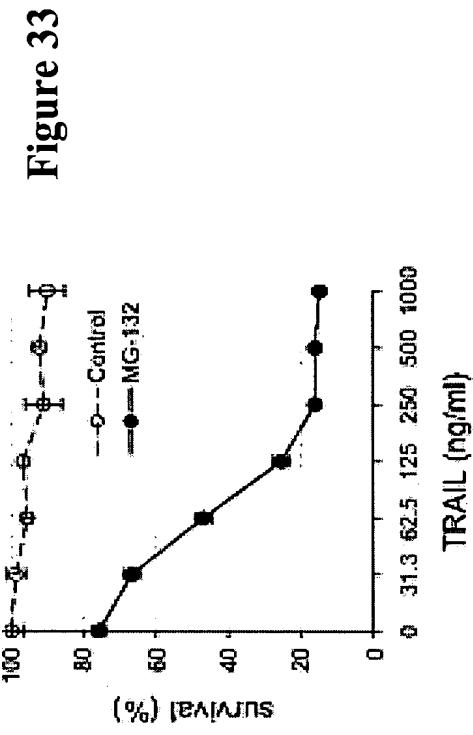
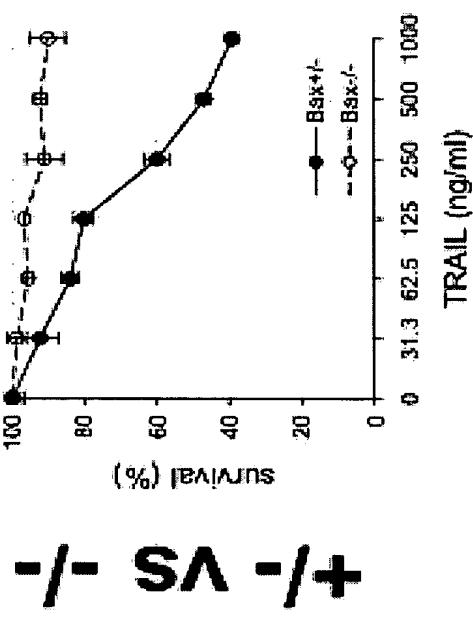


Figure 32

Sensitization of Hct116-Bax-/- to TRAIL by inhibition of the proteasome



Measurement after 24 h, 5 μ M LC, 1 μ M MG-132, 1 nM MG-262

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Figure 34
MG-262 restores the mitochondrial apoptosis pathway

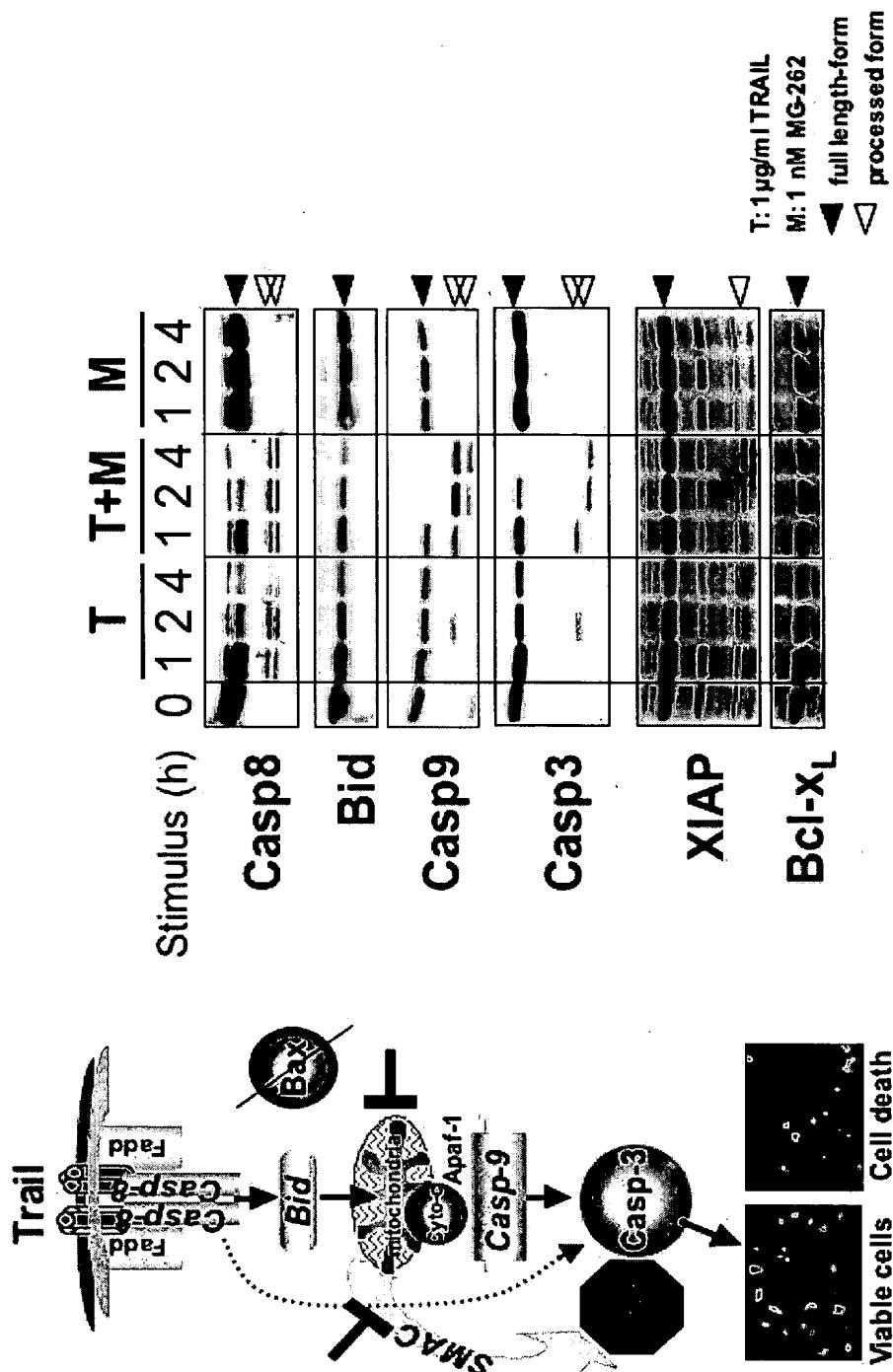


Figure 35

Sequence of DR5'A' heavy chain variable region

AAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGCTGGAAACCCGGGCATCAGTGAA
GCTGTCTGCAGGGCTTCTGGCTACACCTTCACTGACTATACTGACTATACTGGGTAAA
GCAGAGGGTCTGGACAGGGTCTTGAGTGATTGGGTTACCCCTGGAGGGTGGTTA
TATAAAATACAATGAGAAATTCAAGGGCACATGACTGCGGACAAATCCTC
CAACACAGTCTATATGGAGCTTAGTCGATTGACATCTGAAGACTCTGGGTCTATTTC
TGTGCAAGACACGAAGAGGGCATCTATTGACTCTGGGCCAAGGCACCACTCTC
ACAGTCTCCTCA

Amino acid sequence of VH

KVQLQQSGAELVKPGASVKLSCKASGYTFTDYTIHWVKQRSGQGLEWIGWFYPGGGYIK
YNEKFKDRATLTADKSSNTVYMEISRLTSEDSAVYFCARHEEGIYFDYWVGQGTTLTVSS

DNA Sequence of DR5'A' light chain variable region

GACATTGTGATGCCAGTCTCACAGTTCATGTCCACATCAGTGGGAGACAGGGTCA
GCATCACCTGCAAGGCCAGTCAGGATGTGAATACTGCTATAAGCCTGGTATCAACAAAA
ACCAGGGCAATCTCCTAAACTACTGATTACTGGCATCCACCCGGCACACTGGAGTC
CCTGATCGCTCACAGGCAGTGGATCTGGACAGATTACTCTCACCATCAGCAGTGT
GCAGGCTGAAGACCTGGCACTTATTACTGTCAAGAACATTACCACTCCATTACACGT
TCGGCTGGGGACAAAGTTG

Amino acid sequence of VL

DIVMTQSHKFMSTSVGDRVSSITCKASQDVNTAIAWYQQKPGQSPKLLIYWASTRHTGVGPDR
FTGSGSGTDTLTDYTLTISSVQAEDLALYYCQQHYTTPFTFGSGTKL